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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

001838

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: December 8, 1981

SUBJECT: EPA Reg.#464-546, 464-554, PP#1F2508. Garlon 3A, and Garlon 4A Herbicides for use on grasses, forage and hay. Request for tolerances in/on grasses, forage, hay, milk, meat and meat by-products, kidney and liver of cattle, sheep, and goats.
CASWELL#882I Accession#070042, 070043, 070044

FROM: William S. Woodrow, Ph.D WSW 10/29/81 WSW for LDC 1/13/82
Toxicology Branch/HED (TS-769)

TO: Mr. Robert Taylor (25)
Registration Division (TS-767)

Petitioner: Dow Chemical Co.
P.O. Box 1706
Midland, Michigan 48640

Recommendations by Toxicology Branch:

1. The request by Dow Chemical Co. for the following proposed tolerances of the herbicide triclopyr; 3,5,6-trichloro-2-pyridinyl-oxyacetic acid, and its metabolite 3,5,6-trichloro-2-pyridinol is not toxicologically supported:

1000 ppm in or on grasses, forage;
300 ppm in or on grasses, hay;
0.1 ppm in milk;
0.1 ppm in or on meat, fat, and meat by-products (except kidney and liver of cattle, sheep, and goats);
1.0 ppm in or on kidney and liver of cattle, sheep, and goats.

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2. Final validation of an IBT Two-Year Chronic Oral Toxicity Study of Dowco 233 in rats has not been completed (IBT Report No. 621-06138, EPA Accession No. 241901, Submitted 2/29/80).

3. A teratology study in a second species will be required.

4. The 2-year oncogenic evaluation of Dowco 233 in mice should be repeated (the spontaneous incidence of pulmonary tumors precluded adequate interpretation of experimental results, and was suggestive of positive effects).

5. New Toxicity Data Submitted with the Present Report:

   a. Effect of Dowco 233 on Pregnancy of the New Zealand White Rabbit.
   NOEL = 25 mg/kg/day (highest dose tested)
   Core-Minimum Data

   b. Teratogenic Evaluation of Dowco 233 in the Rat.
   NOEL = > 200 mg/kg/day (HDT) (Fetotoxic effects at 200 mg/kg/day; retarded ossification of fetal skull bones at 200 mg/kg/day, and elevated incidence of sternbrae variations at 100 mg/kg/day dam treatment)
   Core-Minimum Data

   c. Mutagenicity Test on Triclopyr (Dowco 233) in Bacterial Systems. Rec-assay and Reversion Mutagenicity Tests - Negative.
   Acceptable Study

   d. Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Dowco 233. No mutagenic potential.
   Core-Minimum Data

   e. Dominant Lethal Evaluation of Dowco 233 in CF-1 Mice.
   Results - Negative (No dominant lethality).
   Acceptable Study

   f. Interim Report: The Metabolism of Dowco 233 following IV and Oral Administration in Rats and Dogs.
   Supplementary Study (May be upgraded upon receipt of final study).

   g. Pharmacokinetic Profile of ^{14}C Dowco 233 Following IV Administration in the Rhesus Monkey. (No significant accumulation in the monkey)
   Core-Minimum Data

   h. Renal Function Studies with Dowco 233 in the Dog and Monkey.
   Renal function in the monkey was unimpaired by Dowco 233, 5 mg/kg/day of Dowco 233 reduced PSP excretion in dogs.
   Core-Minimum Data

i. Acute Oral LD_{50}, Male and Female
Rats Administered Dowco 233
LD_{50} male rats = 729 mg/kg.
LD_{50} female rats = 630 mg/kg.
Core-Minimum Data

j. Subchronic Dietary Feeding Study in Beagle Dogs (Dowco 233)
Phenolsulfonthalein excretion NOEL = 0.5 mg/kg/day
Core-Minimum Data

k. Supplemental Subchronic Dietary Feeding Study in Beagle Dogs (Dowco 233)
Core-Minimum Data
Phenolsulfonthalein (PSP) excretion NOEL (supplementary study) = 0.5 mg/kg/day (more dogs frequently tested should have been incorporated into this study).

l. 28-Day Nasogastric Intubation Test in Rhesus Monkey Using Dowco 233.
No toxic effects.
Core-Minimum Data

m. 2-Year Oncogenic Evaluation of Dowco 233 in the Mouse.
Supplementary Data (Study should be repeated due to unestablished incidence of spontaneous pulmonary tumors in the CDF,/Cox strain of mice used).

6. The signal word and precautionary statements for the GARLON 3A product are satisfactory.

   The signal word and precautionary statements for the GARLON 4A product are not satisfactory. GARLON 4 signal word and precautionary statements must be changed to agree with the GARLON 3A label to include DANGER, plus the proper precautionary statements.

Tolerances Requested:

PROPOSED TOLERANCES FOR THE PESTICIDE CHEMICAL.

Tolerances for combined residues of the herbicide triclopyr, 3,5,6-trichloro-2-pyridinyloxyacetic acid, and its metabolite 3,5,6-trichloro-2-pyridinol, are proposed as follows:

1000 ppm in or on grasses, forage;

300 ppm in or on grasses, hay;

0.1 ppm in milk;

0.1 ppm in or on meat, fat, and meat by-products except kidney and liver of cattle, sheep, and goats;

1.0 ppm in or on kidney and liver of cattle, sheep, and goats.

Recommendations by Residue Chemistry Branch:

"We recommend that the proposed tolerances not be established for the reasons given in conclusions 1c, 2b, 2c, 3a, 3b, 4a and 4c above. Requirements for resolution of these deficiencies are also discussed in the appropriate conclusions above. The petitioner should be informed of these requirements along with our requirement regarding conclusion 1b."

NOTE: (1) In the conclusions by RCB (R. Perfetti, completed but not typed) referred to above, RCB requests that a lactating goat metabolism study be conducted to determine the nature of terminal residue(s) in ruminants. RCB further states that PP#1F2508 Section F should be revised to show tolerance requests of 2000 ppm residues for forage grasses and hay and that appropriate residue tolerance levels of triclopyr requested for the commodities listed should be 0.4 ppm in milk, 1.0 ppm in meat, fat and meat by-products (except kidney and liver) and 5 ppm in liver and kidney.

(2) The petitioner states that the tolerances on forage grasses, grass hay, milk, meat and meat by-products are being proposed to allow current grazing restrictions to be changed to a 3 day withdrawal prior to slaughter and limited grazing of lactating dairy animals.

Garlon 3A and Garlon 4 Herbicide are intended to control woody plants and broadleaf weeds on Rights-of-Way, Industrial Sites and Non-Crop Areas and for use in Forest Site Preparation. Both may be applied by high or low volume ground application, by helicopter as foliar sprays. In addition, basal bark and dormant brush treatments are recommended for Garlon 4, cut surface and injection methods for Garlon 3A.

Garlon 3A Herbicide contains 3 pounds per gallon of triclopyr as the triethylamine salt. Current application rates range from 1/2 to 3 gallons per acre.

Application Rate GARLON 3A per acre	Restrictions for Foliage Applications
More than 1 gallon	Do not harvest treated area for 60 days. Do not graze meat animals for 7 days or graze lactating dairy cows for 30 days after treatment. Withdraw animals from treated feed 3 days before slaughter, unless 120 days post-application.
1/2 gallon to 1 gallon	Do not harvest treated area for 30 days or graze lactating dairy cows for 7 days after treatment. Withdraw animals from treated feed 3 days before slaughter, unless 60 days post-application.
1/2 gallon or less	Do not harvest treated area for 14 days or graze lactating dairy cows for 7 days. Withdraw animals from treated feed 3 days before slaughter unless 30 days post-application.

Garlon 4 Herbicide contains 4 pounds per gallon of triclopyr formulated as the butoxyethyl ester. Current application rates range from 1 pint to 2 gallons per acre.

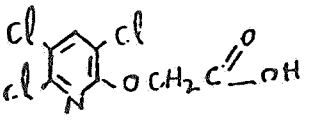
Application Rate GARLON 4 per acre	Restrictions for Foliage Applications
More than 1.5 gallons	Do not harvest treated area for 30 days or graze lactating dairy cows for 7 days after treatment. Withdraw animals from treated feed 3 days before slaughter unless 60 days after treatment.
1.5 quarts to 1.5 gallons	Do not harvest treated area for 14 days or graze lactating dairy cows for 7 days after treatment. Withdraw animals from treated feed 3 days before slaughter unless 60 days after treatment.
1.5 quarts or less	Do not harvest treated area for 7 days after treatment. Withdraw animals from treated feed 3 days before slaughter unless 30 days after treatment.

A. Substance Indentification:

1. Chemical Name: 3,5,6-Trichloro-2-pyridyloxy acetic acid or
3,5,6-trichloro-2-pyridinyloxy acetic acid

2. Synonyms: GARLON 3A HERBICIDE (A.I. = 44.4%) Triclopyr,
GARLON 4 HERBICIDE (A.I. = 61.6%) Triclopyr

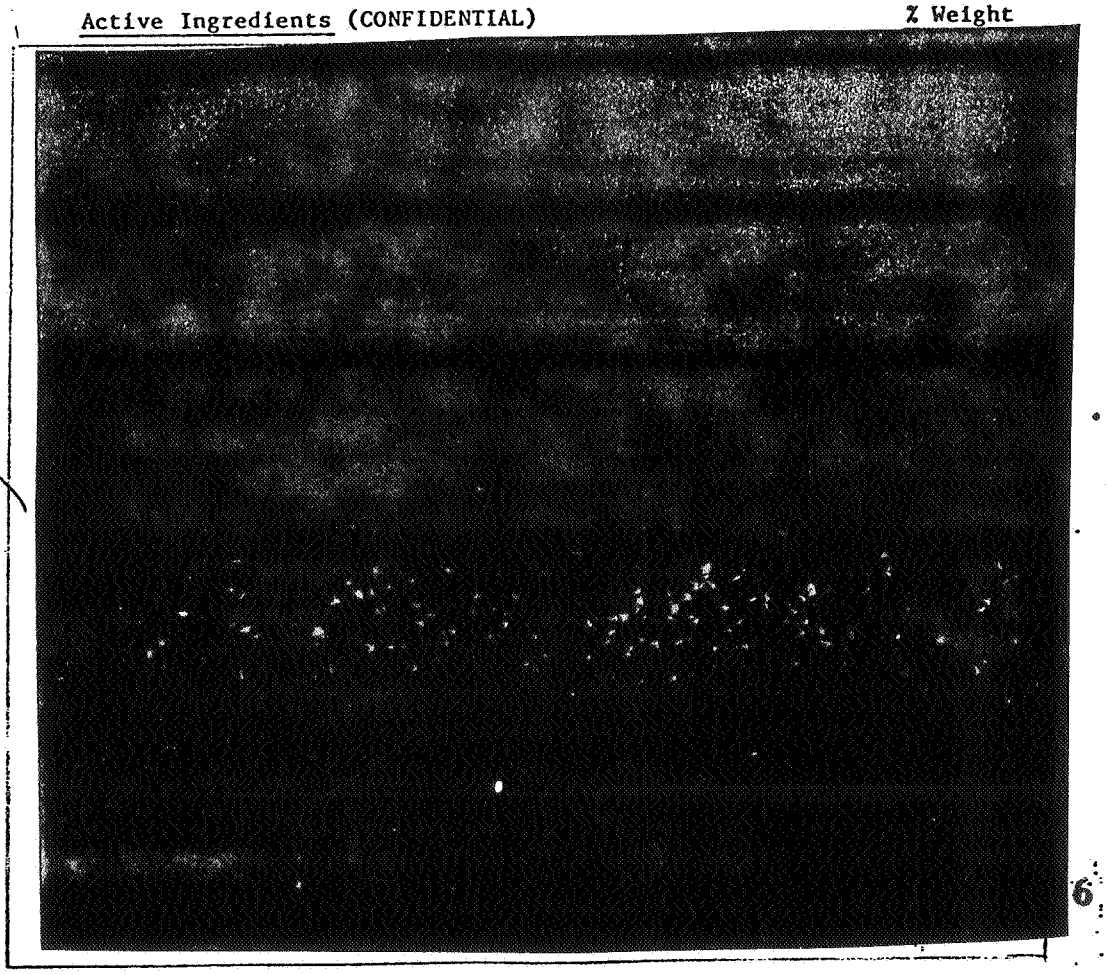
3. Structure:



B. Formulations

Garlon 3A Herbicide

Active Ingredients (CONFIDENTIAL) % Weight



[redacted]

C. Previously Submitted Toxicity Data:

(See 3/2/1978 memo, E. Budd)

Acute Toxicity - Technical

Acute Oral LD_{50}, Rats, Female (= 713 mg/kg) (Tox. Category III), Supplemental Study

Acute Oral LD_{50}, Rats, Male (= 713 mg/kg (Tox. Category III), Supplemental Study

Acute Oral LD_{50}, Cavies, Male (= 310 (236-407) mg/kg, (Tox. Category II), Supplemental Study

Acute Oral LD_{50}, Rabbits, Male & Female (= 550 (300-1000) mg/kg, (Tox. Category II), Supplemental Study

Primary Eye Irritation, Rabbits (Slight corneal injury) (Tox. Category II), Supplemental Study

Primary Skin Irritation, Rabbits (Slight redness) (Tox. Category IV (?), Supplemental Study

Acute Dermal LD_{50}, Rabbits = > 2000 mg/kg (Tox. Category III), Core Study - Minimum Data

Acute Toxicity - Formulation

Acute Oral LD_{50}, Rats, Female = 2140 (1540-2990) mg/kg
Tox. Category III, Core Study - Minimum Data

Acute Oral LD_{50}, Rats, Male = 2830 mg/kg, Tox. Category III, Core Study - Minimum Data

Primary Eye Irritation, Rabbits - corneal damage at 7 days, Tox. Category I, Core Study - Minimum Data

Primary Skin Irritation, Rabbits - necrosis at 72 hours, Tox. Category II, Core Study - Minimum Data

Acute Dermal LD_{50}, Rabbits = > 3980 mg/kg, Tox. Category III, Core Study - Minimum Data

Acute Inhalation LC_{50}, Rats = > 0.8 mg/liter (recalculated)
Tox. Category II, Core Study - Minimum Data

Subacute Toxicity - Technical

Subacute Feeding Study, Rats, 90 Days - NEL = 30 mg/kg/day
Core Study - Minimum Data

Teratology Study, Rabbits - (Negative for terata)
Supplementary Study

Chronic Toxicity - Technical

Reproduction Study, Rats, 3-Generations - NEL $\geq$ 30 mg/kg/day,
Core Study - Minimum Data

Mutagenicity Studies - Technical

Host-Mediated Assay, Mice - Negative, Core Study - Minimum Data

Mammalian Cytogenetic Study, Rats - Negative, Core Study - Minimum Data

Dominant Lethal Assay, Rats - weak positive effect
Core Study - Minimum Data

Acute Toxicity - Possible Metabolite (?) 3,5,6-Trichloro-2-pyridinol

Acute Oral LD_{50}, Rats, Male = 794 (709-889) mg/kg (Tox. Category III), Core Study - Minimum Data

Acute Oral LD_{50}, Rats, Female = 870 (758-1009) mg/kg (Tox. Category III), Core Study - Minimum Data

Acute Oral LD_{50}, Mice, Male = 380 (333-433) mg/kg (Tox. Category II), Core Study - Minimum Data

Acute Oral LD_{50}, Mice, Female = 415 (367-469) mg/kg (Tox. Category II), Core Study - Minimum Data

Subacute Toxicity - Possible Metabolite (?) 3,5,6-Trichloro-2-pyridinol

Subacute Oral, Rats, 90 Days - (NEL = 0.1%), Supplementary Study

(See 5/7/1980 memo, I. Mauer)

Heritable Translocation Test - Interim Report. Results were negative.

(See 4/2/1975 memo, E. Budd)

Teratology study, rat (technical). Major positive developmental defects at 200 mg/kg/day in 2 foetuses generalized edema in a 3rd foetus. NOEL = 100 mg/kg/day.

D. New Toxicity Studies Reviewed in the Present Report:

1. Effect of Dowco 233 on Pregnancy of New Zealand White Rabbits. Tester: Huntingdon Research Center, Huntingdon, Cambridgeshire, England. Sponsor: Dow Chemical Co. August 8, 1979.

Test Chemical: Dowco 233 (Triclopyr-AGR 134832) technical chemical was dissolved in corn oil to provide a 2.5% solution (highest dose). The lower concentration (1.0%) was prepared by diluting the 2.5% solution with corn oil. Formulations were prepared daily.

"The experiment was performed in two parts, Phases A and B. Since it was intended to supplement information obtained in earlier studies by our sponsors(Dow Chemical Co.), only two test groups were employed and dosages were determined by our sponsors."

NOTE: "Phase A and Phase B" were two separate experiments; progeny from Phase A were not employed as parental animals for the Phase B experiment. (Phases A and B were noncuncurrent).

Animals in Phase A were mated September 5, 1978 and killed on October 4, 1978. For Phase B the first animals were mated on January 9, 1979 and the last animals were killed on February 16, 1979.

Sexually mature NZW rabbit does were examined for overt signs of disease, tagged and acclimated to a laboratory environment for 10 days prior to mating.

Does in a weight range of 2.8 to 3.8 kg were mated with males of proven fertilty; those which successfully completed coitus were each injected with 10 i.u. of a lutenizing hormone to ensure ovulation. The day of mating was considered day 0 of pregnancy. On each mating day, mated does were allocated to groups to equalize distribution as far as possible, as to number allocated, source of animals and male to which they were mated.

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Experimental design; Phase A and Phase B are two separate experiments:

Group/ colour code	Treatment mg/kg/day	Concentration of solution		Dosage volume mg/kg	No. of rabbits		Animal number	
		% w/v	vehicle		Phase A	Phase B	Phase A	Phase B
1 : white	Control	-	Corn oil	1	5	18*	1-5	16-33
2 : yellow	Dowco 233, 10	1.0	Corn oil	1	5	17*	6-10	34-50
3 : red	Dowco 233, 25	2.5	Corn oil	1	5	15	11-15	51-65

*Total includes 3 control and 2 test does that died before initiation of dosing.

Animal room controls were set to maintain temperature and relative humidity at 21°C ± 4°C and 60% ± 10% respectively; natural light in the room was supplemented by artificial light between 07.30 and 18.30 hours.

All animals were given free access to Spratt's Laboratory Diet No. 21066 and to tap water.

Dosing by intragastric intubation at 1 ml/kg (2.5% solution - high dose, 1.0% solution - low dose) commenced on day 6 of pregnancy and continued daily up to and including day 18 of pregnancy. Dose volumes for individual animals were calculated or adjusted according to body weight on days 6, 10 and 14 of pregnancy.

Observations: Does were observed daily for obvious changes or signs of reaction to treatment and weighed on days 1, 6, 10, 14, 19, 23 and 29 of pregnancy.

All animals that died or were killed were weighed and subjected to post-mortem examination.

On day 29 of pregnancy the animals were killed by cervical dislocation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs; the ovaries and uteri were examined immediately to determine:

number of corpora lutea;
number and distribution of live young;
number and distribution of embryonic/ foetal deaths;
individual foetal weight; and
foetal abnormalities.

Embryonic/foetal deaths were classified as:

Early - both placental and embryonic remnants were visible at termination;

Abortion - only implantation site scars visible at termination.

Live young were examined then killed. They were weighed, dissected, examined internally for abnormalities and sexed. Where appropriate, young showing abnormalities were examined by alternative procedures (e.g. microdissection, histopathology) to clarify initial observations. Young were skinned, eviscerated and fixed in 74 op industrial methylated spirit; after fixation the heads were sliced through the line of front parietal suture and the brain examined for visible anomalies (e.g. hydroencephaly, hydraencephaly) prior to clearing and staining for skeletal examination.

Structural deviations were classed as major malformations: probably lethal, e.g. amelia, encephaly.

Minor anomalies, e.g. variations of the gall bladder, or upon skeletal examination, e.g. asymmetrical sternbrae.

Variants - Alternative structures occurring regularly in the control population were classed as variants, e.g. an extra pair of ribs, or they may be transient, e.g. unossified sternbrae.

Assessment of results for each litter:

$$\frac{\text{No. of corpora lutea} - \text{No. of implantations} \times 100}{\text{No. of corpora lutea}}$$

= pre-implantation loss

$$\frac{\text{No. of implantations} - \text{No. of live young} \times 100}{\text{No. of implantations}}$$

= post-implantation loss

Group mean values were then calculated from individual observations in two ways:

Mean A - includes all surviving animals that provided evidence of pregnancy, including those aborting or exhibiting total resorption.

Mean B - includes all animals with live young at termination.

For all values expressed as a percentage or ratio values were first calculated within the litter and the group value derived as a mean of individual litter percentages. Statistical analyses were then performed using the litter as the basic sample unit and non-parametric methods since litter values rarely follow a normal distribution.

Statistical analyses were performed to determine compatability of data from Phase A and Phase B regarding body weight changes or litter values.

Results:

1. Parental Animals

NOTE: Tester pooled Phase A and Phase B experimental results.

a) Mortality, exclusions, viable young, resorptions

Resorptions/litter:

Group 1 (Control)	Dam#	Resorptions	% Post-implantation loss
	3	0	0.0
	5	1	10.0
	16	1	20.0
	18	0	0.0
	23	0	0.0
	26	2	20.0
	27	2	16.7
	29	9	64.3
	20	0	0.0
	33	0	0.0
	21	2	15.4
	28	0	0.0
Group 2 (10 mg/kg/day)	6	1	11.1
	7	1	11.1
	8	0	0.0
	9	0	0.0
	10	2	20.0
	34	4	28.6
	36	2	25.0
	37	0	0.0
	38	0	0.0
	39	0	0.0
	42	0	0.0
	43	0	0.0
	48	0	0.0
	49	0	0.0
	50	3	33.3
	44	4	36.4
	47	1	11.1
Group 3 (25 mg/kg/day)	13	0	0.0
	15	3	33.3
	54	1	14.3
	56	0	0.0
	57	2	22.2
	58	0	0.0
	59	0	0.0
	60	2	8.3
	61	0	0.0
	62	3	33.3
	63	0	0.0
	65	4	33.3

Table 1

Group:	1	2	3
Compound:	CONTROL		DOWCO 233
Dose (mg/kg/day):		10	25

Observation	Number of animals in groups 1	2	3	Historical control
Mated and dosed	20†	20‡	20	'20'*
Died, killed or excluded				
(a) Intestinal disorder	3	1	6	2.5
(b) Other	2	2	0	1.2
Non-pregnant	4	2	1	2.0
Total resorption and/or abortion	1	0	1	0.7
With viable young at termination	10	15	12	14.0

†Excludes 3 animals killed before dosing
‡Excludes 2 animals killed bofore dosing
*Historical control from a total of 137 animals employed in 4 studies prior to and 2 studies after performance of current study: values expressed as from a group size of 20 for convenience

Table 1 shows:

An excessive number of parental deaths - due to an enteric disorder, or to other causes.

Resorptions/abortions were affected by treatment.

Number of does with viable young apparently was not treatment related.

It is possible that the greater numbers of parental deaths in the 25 mg/kg/day group could be treatment related.

b) Body weight gains of dams with viable young:

Table 2 Group mean bodyweights

Group: 1 2 3

Compound: CONTROL DOWCO 233

Dose (mg/kg/day): 10 25

GROUP	NO. OF ANIMALS	BODYWEIGHT (g) AT DAY						
		1	6	10	14	19	23	29
1	A = 11	3373	3505	3600	3718	3830	3962	4029
	B = 10	3345	3471	3553	3674	3794	3925	4008
2	A = 15	3336	3484	3595	3694	3768	3913	4067
	B = 15							
3	A = 13	3384	3565	3654	3766	3828	3923	4083
	B = 12	3376	3554	3672	3781	3830	3910	4058

NOTE: Tester separated Phase A and Phase B Group mean body weight data.

Table 2 shows:

Group mean body weights of Phase A experimental dams with viable young did not show treatment effects.

A dose-related retardation of body weight gains for Group B mean values during the latter half of the dosing period (between days 14 and 19 of pregnancy was apparent:

Dosage (mg/kg)	Wt. gain, g.
0	147
10	74
25	49

A slower weight gain recovery after treatment stopped was observed for the higher (25 mg/kg) dams. Both treatment groups completely recovered weight gains by experiment termination.

c) Pregnancy rate - The member of pregnant animals per group and percentage pre-implantation loss per litter were not adversely affected by treatment.

d) Terminal dam autopsy - The most common gross changes observed at post-mortem examination were those suggesting enteric disorder; these changes were not evident among animals that died or were excluded.

2. Litter Data

NOTE: Data for Phase A and Phase B was pooled for the 10 mg/kg dose level; the 0 dose control and the 25 mg/kg dose level data were reported separately for Phase A and B.

Treatment with Dowco 233 apparently did not affect the following litter parameters when control and treated dam data values were compared: litter size, pre and post-implantation losses, litter and mean foetal weights or sex ratios.

Slightly lower mean foetal weights at 25 mg/kg dose levels was considered a consequence of the higher litter size at this dose level; litter weights at 10 and 25 mg/kg were slightly greater than those of controls.

One control animal showed total resorption and one animal at 25 mg/kg aborted.

The incidence of major malformations was comparable for all groups. The incidence of visceral anomalies was lower in test groups than that of controls. A higher incidence of skeletal anomalies in test groups compared to controls did not differ significantly ($p > 0.05$); anomalies were not dose-related. The skeletal anomalies most frequently encountered were ossification and sternebrae irregularities.

Skeletal anomalies compared between groups were not statistically different.

Conclusion: No evidence of pregnancy/teratogenic effects were observed when Dowco 233 was tested in pregnant New Zealand White rabbits. Maternal NOEL = 10 mg/kg/day (retardation of body weight gain during latter half of dosing period).

NOTE: A previously submitted Garlon teratology study in the rabbit reviewed by E. Budd, 3/2/1978, was classified as supplementary data. The applicant reported maternal mortalities of 31, 57 and 53% for dams treated with 25, 50 or 100 mg/kg//day, respectively. The actual mortalities were calculated by Budd to be 25, 80 and 47% for the 25, 50 and 100 mg/kg/day groups, respectively. In addition, 44% of the control animals died. The reviewer (Budd) was not able to interpret the data due to the high mortalities found and due to inconsistencies in the data, and to animals unaccounted for. No data was presented on numbers of foetuses, whether absorptions occurred, and no statements about the occurrence of terata or any reproductive parameters was given.

2) Teratogenic Evaluation of Dowco 233 [(3,5,6-Trichloro-2-Pyridyloxy) Acetic Acid] in the Rat.

Performed by the Dow Chemical Co. P.O. Box 6851 Indianapolis, Ind. 46268. 3/5/79.

Test Material: Dowco 233 (Agr 134,832) was supplied by the Dow Ag-Organics Dept.; 98.5% pure technical chemical. The chemical was prepared for oral administration each day prior to use as a suspension in aqueous 0.5% hydroxy propyl methyl cellulose HG 15,000 cps (Dow METHOCEL) at a concentration of 50 mg/ml. Control animals received Methocel at a volume equivalent to that administered to the highest dosage level group. The compound was administered orally by gavage. Doses were calculated on individual body weights on the 6th day of gestation.

Preliminary range finding study:

Six groups of 5 mated Sprague-Dawley female rats each with body weights of 205-215 g were used. The test compound was administered once daily on days 6-15 of gestation.

Group	Dowco 233 mg/kg/day
1	0 (control)
2	25
3	50
4	100
5	200
6	400

Dams were killed on day 20 of gestation. Fetuses were removed by cesarian section, examined for gross external anomalies, and weighed by sex. The number and position of viable fetuses and resorption sites were recorded. Corpora lutea were counted. Detailed examination was performed only on grossly malformed fetuses. Rats dying spontaneously were subjected to necropsy.

Teratology Study

Test Material: Same as above

Four groups each consisting of 25 virgin female Sprague-Dawley rats with initial group mean body weights of 217-221 g were used. Females were mated with males of known fertility; day 0 of gestation was determined by the presence of sperm in vaginal smears. Bred females were assigned randomly to treatment groups. Oral administration of the test compound once daily by gavage began on day 6 and continued through day 15 of gestation (the oral route would be the most likely route of human exposure to the herbicide).

Group	Dowco 233 (mg/kg/day)
1	0 (control)
2	50
3	100
4	200

On day 20 of gestation, females were killed and fetuses were removed by cesarian section. Each fetus was examined for external anomalies - the following data were recorded:

1. position of the fetus in utero
2. number of live fetuses
3. number of resorptions
4. number of corpora lutea
5. individual fetal weights and sex

Two thirds of the fetuses from each litter were preserved in 95% ethanol, stained and examined for skeletal abnormalities. The remaining one-third of each litter was preserved in Bouin's fixative and examined for soft tissue abnormalities. Skeletal and soft tissue examinations were done with the aid of a dissecting microscope.

Body weights and food consumption - Body weights and food consumption data were collected on days 0, 6, 16 and 20 of gestation.

Clinical observations - All rats were observed daily during the testing period.

Statistics - Statistical evaluation of adult body weight gains and food consumption, number of corpora lutea, resorptions, implantations and viable fetuses was made by one way analysis of variance - Fetal weights were analyzed with a nested analysis of variance.

Multiple comparisons between control and treated groups were then made according to Dunnett's procedure. The incidence of fetal anomalies among litters was analyzed by the Fisher exact test. In all analyses the level of significance chosen was $p < 0.05$.

Results:

Preliminary Range Finding Test - No overt toxic signs were observed when pregnant rats were treated with 25, 50, 100, 200 or 400 mg/kg/day of test compound following the first two doses; succeeding doses elicited severe discomfort intermittently in all treated dams. One rat each from the 100 and 200 mg/kg/day groups exhibited periods of dyspnea, which may have been related to very heavy salivation. All treated dams had rough, dry hair.

Six spontaneous deaths occurred in this study; 5 dams receiving 400 mg/kg/day died after 3 to 5 doses. Necropsies revealed multiple, severe gastric erosion in 4 of 5 of the dams. One dam from the 200 mg/kg/day dose level died on test day 16; necropsy revealed thickened urinary bladder walls, due probably to urinary calculi. All of the rats were pregnant and the embryos appeared to have been alive at the time of the dam's death.

Body weight gains for the 200 mg/kg/day group were lower than controls during treatment and post-treatment periods; food consumption for this group was depressed only during the treatment period. Numbers of viable fetuses were markedly decreased in the same group, whereas numbers of resorptions were not increased. Pup weights were not affected by treatment of dams with Dowco 233. All fetuses were normal externally.

Teratology Study - Maternal toxicity resulting from treatment with Dowco 233 in general was dose-related and transient. Excessive salivation was noted in all treatment groups, apparently only when the test compound was deposited in the mouth, and was minimal when the gavage needle was carefully rinsed prior to treatment. Rough hair and excessive shedding was observed in all treatment groups. Most of the rats in all treatment groups exhibited abdominal stress by stretching out on the bottom of the cage and closing eyes. Such behavior occurred immediately after treatment and continued approximately 15 minutes in the group receiving 50 mg/kg/day, and up to 1 hour for the high dose level groups. Some rats occasionally displayed dyspnea accompanied by salivation. Tremors of the head and neck were noted in some rats from all treatment groups, which occurred after the 8th, 9th and 10th doses and lasted approximately 5 minutes.

No signficant decrease in food consumption occurred at any treatment level during the post-treatment period. Neither body weight gain nor food consumption was affected in rats dosed with 50 mg/kg/day. Body weight gains for rats scheduled to receive 100 mg/kg/day were significantly lower than controls, whereas body weight gains during treatment were significantly lower only for rats receiving 200 mg/kg/day. Overall weight gains for both treatment groups were significantly lower than for controls. Food consumption was significantly decreased during the treatment period in the groups receiving 100 or 200 mg/kg/day.

Data collected at cesarian section - Data collected on gestation day 20 during cesarian section indicated treatment of dams with 50, 100 or 200 mg/kg/day of test compound during days 6 through 15 of gestation did not affect numbers of implantations, corpora lutea, or pup sex ratios. A slight, but not significant decrease in pup weight occurred at the high dose level; the resorption rate was increased (but not significantly) at all dose levels. The mean live litter size at all dose levels was not affected by treatment.

Minor skeletal variants such as retarded ossification of sternbrae, vertebrae and metacarpels were observed in fetuses from the control and treatment groups. Such variants were not increased among Dowco 233 treatment groups. However, retarded ossification of skull bones was significantly increased among litters of dams receiving 200 mg/kg/day. Other minor variants observed in fetuses from control and treated groups included sternal defects, accessory lumbar ribs, dilated renal pelvis and distended ureters. None of these were significantly increased in the treated groups. External anomalies included kinky tail in a fetus from a control dam and a generalized edema in a fetus from a dam that received 200 mg/kg/day. Also, two fetuses from the 200 mg/kg/day group had major malformations consisting of cleft palate and brachycephaly; skeletal examinations of these two fetuses revealed a poorly ossified and malformed pelvic girdle, malformed scapulas and malformed bones of the fore and hind limbs. The ribs were short and clubbed.

Thus, various degrees of maternal toxicity resulting from treatment of pregnant rats with high doses of Dowco 233 was shown by decreased body weight gains and food consumption, evidence of discomfort after treatment, occasional dyspnea and tremors and excessive salivation. Maternal toxicity occurred at all dose levels; however, toxicity to the fetus was observed largely at the highest dose level of 200 mg/kg/day. Fetotoxicity was apparent in retarded ossification of the skull bones at the 200 mg/kg/day dose; it would also appear that treatment of dams with 100 mg/kg/day resulted in elevated incidence of defects in fetuses (includes unossified, hemi-, malaligned, misshapen, split and fused sternebrae). Apparently, doses of 50 mg/kg/day, although mildly toxic to the dam, seemed to produce no effect to the developing conceptus.

Maternal LEL = 50 mg/kg/day (LDT)

NOEL for fetotoxicity = 50 mg/kg/day

Negative for teratogenicity at 200 mg/kg/day (HDT).

Classification: Core-Minimum Data

3) Mutagenicity Test on Triclopyr (Dowco 233) in Bacterial Systems.

Performed by the Institute of Environmental Toxicity Kodaire-Shi, Tokyo, Japan. Sponsor: Dow Chemical Co. April 28, 1980

Test Material: Triclopyr (Dowco 233) (3,5,6-trichloro-2-pyridinyl-oxy-acetic acid) 98% pure.

Mutagenic potential of the test compound was examined in rec-assay and reversion tests.

a. Rec-Assay (recombination repair system). Rec-assay mutant (H17) and recombination repair deficient mutant (M45) of B. subtilis were used for investigation of triclopyr mutagenic potential. The mutants were preserved at - 80°C. Mutants were melted and stretched on the same B-11 agar culture with a small B-11 agar culture with a small sized pipette, starting from different points. 0.2 ml of test compound diluted with DMSO was soaked in a round filter paper and placed on the culture plate, covering the starting points of streaks and incubated overnight at 37°C. The length of the inhibitory area was measured for comparison of Rec-inhibition between Kanamycin used for negative control, and Mitomycin C used for a positive control.

b. Reversion Test - Salmonella TA-98 and TA-100 histidine deficient mutant strains were used. The mutants (stored at - 80°C were melted, washed by centrifugation and floated in equal volumes of 0.15 M phosphate buffer at pH 7.0.

The metabolic activation system was prepared by IP administration of 500 mg/kg of Aroclor to SD male rats. After 4 days of fasting, which began the 4th day after dosing, the animals were sacrificed and livers were removed. The livers were cooled at 5°C in 0.5 M Kcl, homogenized and centrifuged at 9,000 xg. 1.0 ml aliquots of the supernatant fraction (S-9 mix) were used as follows:

0.3 ml liver homogenate supernatant
8 mM $Mgcl_2$
33 mM Kcl
5 mM Glucose-6-phosphoric Acid
4 mM $NADP^+$
100 mM Na-phosphoric Acid
bu - ferosolution, pH 7.4

0.1 ml bacterial suspension, 0.1 ml sample solution and 0.5 ml of S-9 mix were added to 2 ml of soft agar solution including 50 uM Biotin and 50 uM histidine; the mixture was shaken thoroughly and placed on minimum agar culture plates. Plates were incubated at 37°C and reversion colonies were then counted. 2-amino anthracene, [2-(1-furyl)-3(5-nitro-2-furyl)acrylamide] was used as a positive control.

Results:

a. Rec-assay - No growth inhibition was measured for the repair competent or the repair deficient bacterial strains employed.

b) Reversion Test - Positive control chemicals (2-amino anthracene) and AF-2 [2-(1-furyl)-3(5-nitro-2-furyl) acrylamide] activated by S-9 mix, included marked reversions to prototrophy in both histidine - deficient Salmonella strains employed. In contrast, no increase in reversion colony counts were found when the test compound was similarly tested against these organisms.

Conclusions: Dowco 233 test compound showed negative results in the Rec-assay and Reversion mutagenicity tests.

Acceptable Study

4) Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Dowco 233 (No other identifying information available).

Test Material: Dowco 233. The solvent employed was DMSO.

Bacterial tester strains employed were TA-1535, TA-1537, TA15-38, TA-98 and TA-100. The test compound test used in bacteriostatic tests were: 10,000, 1000, 100 and 10 ug per agar well. In the mutation test, these tester strains were exposed to the above concentrations of Dowco 233. The following positive control agents were employed:

sodium azide - 5 or 500 ug/plate;
4-nitro-o-phenylene diamine at 500 ug/plate;
2-amino anthracene at 2 ug/plate, or;
2-acetyl-aminofluorene at 20 ug/plate

Data recorded included the mean number of revertant colonies and individual plate counts of revertant colonies for all the Salmonella tester strains. Data collected determined tester strain variability, compared sterility and positive control chemical mutability checks.

Results:

The high dose level of Dowco 233 (10,000 ug/plate) proved toxic toward some of the tester strains of bacteria.

Somewhat erratic results were found with tester strains 1537 and 1538 in the presence of metabolic activation, and for this reason these assays were repeated. However, in no instance were numbers of revertant colonies suggestive of mutagenic effects.

An increase in revertant colony numbers of tester strain 1535 was observed at the top dose level of Dowco 233 in the absence of metabolic activation. No substantial increase in numbers of revertant colony numbers were found when assay with tester strain 1535 was repeated twice.

Conclusions: Dowco 233 did not show any evidence of mutagenic potential using the Salmonella tester strains TA-1535, TA-1538, TA-1537, TA-15, TA-98 and TA-100 tested at dose levels of 10,000 ug, 1000 or 10 ug/plate.

Acceptable Study

5) Dominant Lethal Evaluation of Dowco 233 in CF-1 Mice. Tester: Dow Chemical Co. Toxicology Research Laboratory, Health and Enviromental Science, Midland, Michigan (No other identifying information).

Test Material: Dowco 233 (technical) 99% pure. Dowco 233 was shown to be stable in mixed diets during a 4 week period. The test compound was administered to male mice via the diet. A 0-2% compound - feed pre mix was prepared every other week using a ball mill. Compound - diet mixtures for each dosage group were prepared fresh weekly. The concentration of Dowco 233 in the diet was adjusted weekly according to the rate of food consumption and body weight gain of the animals to maintain the dosage on a mg/kg/day basis.

Reference diet samples (one dose level/week) and pre mix (performed weekly) were saved for test chemical analysis.

Groups of 30 male mice were maintained on diets containing Dowco 233 levels of 0, 3, 15 or 70 mg/kg/day for 9 consecutive weeks. (A 70 mg/kg/day top dose level was used in a previous rat dominant lethal study; mice display a lower LD_{50} compared to rats).

Immediately following treatment each male in the study was mated to four untreated mature virgin females for 7 consecutive days. Two of these females selected at random were removed for a heritable translocation test, and the remaining two females were placed in cages and held for the dominant lethal study. Four other untreated females were then housed with each of the same males for an additional week (period 2 of breeding); which was followed by a similar female distribution for a heritable translocation test and the present dominant lethal study.

A separate group of 30 male mice was employed as a positive control group that received triethylene-melamine (TEM). The TEM was administered by a single I.P. injection of 0.3 mg/kg in 0.9% Nacl. Twenty-four hours following the TEM dose, these males were bred to unexposed virgin females using the same mating schedule described previously.

General Observations - All mice were observed at least once weekly for toxic signs and abnormal behavior. Body weights and food consumption for the F_0 males were recorded weekly during the 9 weeks treatment period.

Ten days after the last day of cohabitation, the females from the dominant lethal portion of the study were sacrificed. The uterus was exposed by mid-line incision in the abdominal wall, and the number of live and dead implants were counted. The uteri of apparently non-pregnant animals were stained with a 10% solution of sodium sulfide and examined for evidence of early resorption sites. The number of resorption sites (if any) showed by staning was recorded and used in the calculation of the resorption rate.

staining

Indices:

Male Fertility Index:

$$\frac{\text{No. of males with at least one implanted female X100}}{\text{No. males housed with females}}$$

Resorption rate:

$$\frac{\text{No. resorption sites X100}}{\text{No. of implantation sites}}$$

The resorption rate was calculated for each litter, and the mean value from both females was calculated for each male for each breeding period.

Statistical Analysis - Body weight, food consumption, litter size, and the number of implantations were evaluated by a one-way analysis of variance. Difference between experimental groups and the controls for body weights and food consumption were examined using Duncan's test, using the TEM treated males as a second control group. This was done because TEM was injected only on the last day of the 9 week treatment period. Differences in litter size and the number of implantations between treated and control groups were examined using Dunnett's test. The fertility index was analyzed using the Fisher's Exact Probability test. the resorption rate was analyzed by using the Wilcoxon test.

Statistical outliers ($P < 0.02$) were excluded from the calculations of food consumption. The level of significance for all other calculations was analyzed using the male as the experimental unit.

Results: No significant toxic effects were observed among the F_0 males treated with 3, 15 or 70 mg/kg body weight via the diet for a period of 9 consecutive weeks.

Statistically significant lower body weights were seen at various times when the 3 mg/kg/day group was compared to the TEM group, but no differences were observed when this treatment group was compared to untreated control values. No differences in body weights were noted at the higher dose levels when values were compared with the TEM positive, or the untreated control groups.

Statistically significant increases in food consumption for the 70 mg/kg/day Dowco 233 treatment group was noted during the eighth week, compared to untreated controls, and significant food consumption increases were noted for this group during the fifth and eighth week when compared to the TEM positive control group. There were no differences at either the 3 or 15 mg/kg/day dose levels when compared to either control. Inconsistencies in food consumption values indicated that these effects were not treatment-related.

Some deaths occurred among untreated F_0 females mated to the F_0 males. Two females from the group mated to control males died after the end of the mating period, six from the group mated with 15 mg/kg/day males, and three among those mated to males treated with 70 mg/kg/day died.

These deaths were considered spontaneous and not related to treatment.

The following tables serve to illustrate the effect of Dowco 233 on reproductive parameters designed to measure dominant lethal effects:

TABLE I

Fertility Index of Male Mice Treated with Dowco 233 via the Diet, Expressed as Percent.

		Dose, mg/kg/day				
		0	3	15	70	TEM**
Post Exposure Breeding						
Week 1	(%)	100 (29/29)	90 (27/30)	97 (29/30)	93 (28/30)	93 (29/30)
Week 2	(%)	97 (28/29)	100 (30/30)	93 (28/30)	100 (30/30)	93 (28/30)

*Fertility Index (%) = $\frac{\text{\# Fertile Males}}{\text{\# Males housed with females}} \times 100$

**T.E.M. was administered by I.P. injection

No values were significantly different from the control by the Fisher's Exact Probability Test, $p < 0.05$.

TABLE II

Average number of implantations and resorptions in unexposed females bred to male mice treated with Dowco 233 via the diet.

	Dose, mg/kg/day				
Week 1	0	3	15	70	TEM**
Av. # implantations	13 ± 2	13 ± 3	12 ± 3	12 ± 4	11 ± 3
Av. # resorptions	1.2 ± 0.9	1.0 ± 0.8	1.2 ± 1.1	1.2 ± 0.9	5.5 ± 2.3
Week 2					
Av. # implantations	12 ± 3	13 ± 2	13 ± 2	13 ± 2	9 ± 2*
Av. # resorptions	0.4 ± 0.4	0.8 ± 1.3	0.6 ± 1.3	0.7 ± 0.7	5.7 ± 2.0

NOTE: 1) Values represent means ± standard deviations. The average number of implantations or resorptions for the two female mice bred to each male was calculated. Group means were then calculated from the averaged values.

**Single 0.3 mg/kg I.P. injection with TEM.

*This value was statistically significantly different from the control value by Dunnett's test, $p < 0.05$.

2) The resorption data shown above were analyzed as resorption rates, which are shown in Table 3 below.

Historical control data for CF-1 female mice:

Total control groups - 31
Total no. of litters - 801

	Mean*	Range
No. of implantations/dam:	12.4 ± 1.0	10-14
No. of resorptions/litter:	1.5 ± 0.5**	0.7 to 2.0

$\frac{\text{Resorptions}}{\text{Implantations}}$ x 100 = % of litters with resorptions = 13.5 ± 113
range 39 to 85

*Mean of the 31 individual control groups.

**Mean of 14 control groups and 404 litters.

TABLE 3

Average resorption rate (%) in unexposed female mice bred to male mice treated with Dowco 233 in the diet.

	Dose, mg/kg/day				
Post-exposure breeding	0	3	15	70	TEM*
Week 1	9 ± 7	8 ± 6	11 ± 10	10 ± 8	53 ± 23**
Week 2	4 ± 5	6 ± 10	5 ± 4	7 ± 10	65 ± 23**

*A single 0.3 mg/kg dose of TEM used.

Resorption rate = $\frac{\text{No. resorptions X100}}{\text{No. implantations}}$

The resorption rate for the two females bred to each male mouse was calculated. Group means were then calculated from these averaged values. Values are reported as mean ± standard deviation.

**Significantly different from the control mean by a modified Wilcoxon test, $p < 0.05$.

TABLE 4

Average litter size of untreated female mice bred to male mice treated via the diet.

	Dose, mg/kg/day				
	0	3	15	70	TEM*
Post-exposure Breeding					
Week 1 - Day 1	10.7 ± 3.1	10.7 ± 3.3	9.9 ± 3.9	10.6 ± 2.6	4.9** ± 3.6
Day 21 (weaning)	9.8 ± 3.4	9.4 ± 3.9	7.9 ± 4.7	9.1 ± 3.8	4.5** ± 3.8
Week 2 - Day 1	11.9 ± 2.6	12.2 ± 2.3	11.6 ± 2.9	11.0 ± 2.4	3.5* ± 2.5*
Day 21 (weaning)	11.7 ± 2.4	11.9 ± 2.6	11.5 ± 2.9	10.9 ± 2.4	3.4** ± 2.6

*TEM administered I.P. as one 0.3 mg/kg/dose

Significantly different from the control means by Dunnett's test, $p < 0.05$.

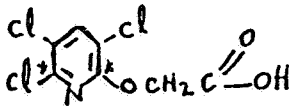
Conclusions - Treatment of CF-1 male mice with 3, 15 or 70 mg/kg/day for 9 weeks via the diet did not induce dominant lethality. No effects on body weight or food consumption were noted. Reproductive values for treated groups of mice were comparable to untreated controls. The average number of implantations for all groups was 12 to 13, which agrees with a historical incidence of 12.4. Resorption rates for all treated groups lie within the range of control values.

Treatment with TEM, a known mutagenic positive control agent, resulted in increased resorption rates.

Acceptable Study

6) Interim Report: The Metabolism of Dowco 233 following IV and Oral Administration in Rats and Dogs. Tester: Dow Chemical Co. Health and Consumer Products Pharmacokinetic/Metabolism Group, Midland, Michigan. April 6, 1976.

Test Material - Dowco 233 technical chemical, or technical chemical labeled ^{14}C:



labeled at the 2 and 6 positions.

specific activity of 1.18 mCi/mmoles (4.58 uCi/mg)

Thin layer chromatography indicated that 98% of the ^{14}C Dowco 233 was located at the position of unlabeled Dowco 233, in the following TLC systems:

benzene: acetic acid
benzene: methanol
benzene: dioxane: formic acid

The ^{14}C activity of dosing solutions was determined by liquid scintillation counting of weighed aliquots.

The study objectives were to determine the distribution and excretion of ^{14}C following a single IV dose of ^{14}C Dowco 233, and to determine the absorption of ^{14}C Dowco 233 following a single oral dose of ^{14}C Dowco 233; evaluated in both rats and dogs.

Twenty rats were given a single IV dose of ^{14}C Dowco 233 at dose levels of 1-100 mg/kg. Three rats were given a single oral dose at 100 mg/kg. Each of 3 dogs was given a single IV dose of ^{14}C Dowco at 0.5, 5 or 20 mg/kg and 2 dogs were given single oral doses of 0.5 or 20 mg/kg.

The dose volume administered to rats varied from 1-5 ml/kg and 1-1.3 ml/kg in dogs. The average radioactive dose was 5.6 uCi/kg in rats and 1.4 uCi/kg in dogs.

Male and female Sprague-Dawley rats were treated by the oral route; they were deprived of food 12 hours prior to treatment. All the rats were implanted with indwelling jugular cannulas 48 hours prior to dosing. Animals were housed in metabolism cages designed for separate collection of urine and feces; some were housed in special cages permitting expired CO_2 to be trapped. Following administration of ^{14}C Dowco 233, 100 ul samples of heparinized blood were obtained through the cannulas at various time intervals until ^{14}C activity in blood plasma could no longer be detected. Feces and voided urine were collected at 8 hour intervals and frozen until assayed for ^{14}C activity.

and stored frozen for ^{14}C analysis: liver, kidney, heart, lungs, lean muscle, perirenal fat and intestinal fat. Brain thymus and adrenals were obtained from the three rats used in a probe study.

Pure bred male Beagle dogs were used in the study. Orally treated animals were deprived of food overnight prior to dosing. Available feces samples were collected from the cage floor several times per day. Renal clearance of ^{14}C Dowco 233 was determined by collecting urine samples from the dogs at 3 hour intervals by the use of sterile catheters.

Urine sample collection longer than 3 hours duration was collected through a drain in the cage floor. At termination the dogs were killed and the following organs and tissues were collected and stored frozen for ^{14}C assay: kidney, liver, urinary bladder, lungs, heart, adrenals, thymus, perirenal fat, subcutaneous fat, lean muscle, brain, gall bladder and gall from the gall bladder. Table 1 summarizes experiments:

TABLE 1. Summary of Experiments Carried Out with ^{14}C-DOWCO* 233 in Rats and Dogs

Species	Sex	Dose Level (mg/kg)	Route of Administration	Blood Plasma	RBC	Bile	CO_2	Urine	Feces	Tissues	^{14}C Balance	Comments
Rat	M	20	IV	+	-	-	+	+	+	+	Yes	Probe study
Rat	M	20	IV	-	-	-	+	+	+	+	Yes	"
Rat	M	100	IV	+	+	+	+	+	+	+	Yes	"
Rat	M	5	IV	+	-	-	-	-	-	-	No	No blood samples
Rat	F	5	IV	-	-	-	-	-	-	+	No	obtained
Rat	M	20	IV	+	-	-	-	-	-	-	No	
Rat	F	20	IV	+	-	-	-	-	-	-	No	
Rat	M	5	IV	+	+	-	-	+	+	+	Yes	
Rat	F	5	IV	+	+	-	-	+	+	+	Yes	
Rat	M	20	IV	+	+	-		+	+	+	Yes	
Rat	F	20	IV	+	+	-	-	+	+	+	Yes	
Rat	M	50	IV	+	+	-	+	+	+	+	Yes	Depressed urine
Rat	F	50	IV	+	+	-	+	+	+	+	Yes	flow
Rat	M	100	IV	+	+	-	+	+	+	+	Yes	
Rat	F	100	IV	+	+	-	+	+	+	+	Yes	
Rat	F	100	IV	+	+	-	+	+	+	+	Yes	
Rat	M	100	Oral	+	-	-	+	+	+	+	No	Absorption study
Rat	M	100	Oral	+	-	-	+	+	+	+	No	"
Rat	M	100	Oral	+	-	-	-	+	+	+	No	"
Rat	F	50	IV	+	-	-	-	-	-	-	No	
Rat	F	50	IV	+	-	-	-	-	-	-	No	
Rat	M	1	IV	+	-	-	-	-	-	-	No	Low plasma ^{14}C
Rat	F	1	IV	+	-	-	-	-	-	-	No	
Dog	M	0.5	IV	+	+	-	-	+	+	+	Yes	
Dog	M	5	IV	+	+	-	-	+	+	+	Yes	
Dog	M	20	IV	+	+	-	-	+	+	+	Yes	
Dog	M	0.5	Oral	+	-	-	-	+	+	+	Yes	Absorption study
Dog	M	20	Oral	+	-	-	-	+	+	+	Yes	"

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Analytical Procedures - All ^{14}C determinations were made by scintillation counting. All counting rates were corrected for background and corrected to dpm for calculations. Blood plasma ^{14}C was determined following centrifugation by transferring plasma into tared scintillation vials and counting in 10% water in Aquasol. Urinary ^{14}C was determined directly on gravimetric aliquots; each sample analyzed in triplicate. Tissues and organs from rats were pulverized in a hammer mill at liquid N_2 temperature - 3 portions of each sample were counted directly in an Aquasol suspension. Four replicates of each tissue and organ sample from dogs were oxidized and resulting CO_2 trapped in ethanolamine for counting. Samples of RBC from dogs and rats were oxidized directly and aqueous homogenates of feces samples were prepared prior to oxidization.

Calculation - A net counting rate at least 10% of the background counting rate (^{14}C) was considered significant, which was equivalent to about 3 dpm. Therefore, those samples with a net counting rate of less than 3 dpm were judged to contain less than measurable quantities of radioactivity. When this level of 3 dpm was applied to each individual tissue sample, the corresponding limiting value in ug of Dowco 233/g of tissue was dependent on the specific activity of the administered ^{14}C Dowco 233 and on the quantity of tissue represented by that counting sample.

Results (interim)

1) Probe Study - Two male rats were injected with 20 mg/kg ^{14}Dowco 233 each IV, and one rat was treated similarly with 10 mg/kg. The average overall ^{14}C recovery was 93.2 $\pm$ 2.2%. From 80 to 90% of the administered ^{14}C activity was excreted in the urine, while the feces contained 1 to 4% of the ^{14}C activity. Negligible quantities of ^{14}C were detected in expired CO_2 at either dose level. No significant quantities of ^{14}C were detected in tissues at the 20 mg/kg dose level. At the 100 mg/kg dose level the adrenals contained ^{14}C equivalent to 6.9 ug Dowco 233/g of tissue. Periodically sampled bile showed concentrations of ^{14}C 2 to 3 times higher than in blood plasma. Blood plasma ^{14}C concentrations indicated possible dose-dependent delay in ^{14}C clearance at the higher dose level.

2) Main Studies (Rats) - Following single IV or oral doses of ^{14}C Dowco 233 at 5, 20, 50 or 100 mg/kg, 92.1 $\pm$ 3.1% of radioactivity was recovered. Dose related trends in the routes of excretion seemed apparent; the average percent of ^{14}C excreted in the urine decreased from 90.8% at 5 mg/kg to 83.0% at 100 mg/kg; while ^{14}C in feces increased from 1.1% at 5 mg/kg to 2.9% at 100 mg/kg.

   Following an oral dose of ^{14}C Dowco 233 at 100 mg/kg, rats excreted an average of 74.1% $\pm$ 4.4% of the administered radioactivity in the urine at 32 hours, while 78.2% of radioactivity was excreted at 32 hours following an IV dose at 100 mg/kg.

Tissue Concentration (Rats) - The following table illustrates tissue concentrations of ^{14}C Dowco 233:

Average Concentration of ^{14}C as Microgram Equivalents of ^{14}C-DOWCO 233 per Gram of Tissue in Rats Following IV or Oral Administration of ^{14}C-DOWCO 233**

	IV Administration				Oral Administration
Dose Level (mg/kg)	5	20	50	100	100
Rat Numbers	8,9	10,11	12,13	14,15,16	17,18,19
Hours After Dose	52	52	72	72	32
Plasma	0.014	0.09	0.12	0.55	1.8
Liver	0.05	0.26	0.61	2.1	10.4
Kidney	0.12	0.36	<0.19	<1.2	6.4
Heart	<0.04	<0.12	<0.28	<1.1	3.0
Lung	<0.03	<0.11	<0.30	<1.0	4.0
Muscle	<0.02	<0.13	<0.30	<0.6	2.1
Fat**	<0.06	0.49	<0.20	7.7	7.2
Carcass***	<0.05	<0.12	<0.42	<1.2	N.A.

*The "less than" sign (<) indicates an average sample concentration of ^{14}C below the measurable limit of radioactivity. See text for explantation of the resulting lower limit of detectable concentration of ^{14}C-DOWCO 233.

**Average for both intestinal and perirenal fat.

***Remaining carcass after removal of above organs and tissue samples.

N.A. = not analyzed.

The ^{14}C tissue concentration for both IV and orally dosed rats was in liver and fat tissue - the concentration increasing with increasing doses. The increases were approximately proportional to dose levels; however, interpretation is complicated by the fact that measurements at the 5 and 20 mg/kg levels were made at 52 hours post dosing, while measurements at the 50 and 100 mg/kg levels were made at 72 hours. The table above indicates that all tissues containing measurable quantities of ^{14}C showed higher concentrations than did blood plasma, which indicates a tendency for ^{14}C Dowco 233 to concentrate in tissues relative to plasma.

Blood Plasma (Rats) - Semi logarithmic plots of ^{14}C concentration in rat blood plasma calculated as ug Dowco 233 VS time indicates a dose dependent trend in the clearance ^{14}C from blood plasma. The low dose level of 1 mg/kg showed a first order decrease with a half-life of approximately 1 hour. Dose levels above 1 mg/kg initially show nearly saturated rates of clearance which gradually changes into a first order decrease with a half-life of 1-2 hours. A semi-logarithmic plot of ^{14}C concentration in blood plasma following oral doses to 3 rats of 100 mg/kg each indicates a very rapid absorption shown by the appearance of peak plasma levels at 0.5 hr. post administration. Plasma levels then remained high for about 16-20 hours, at which time the clearance rate shifted to first order decreases with a half-life of about 1.5 hours. 100 mg/kg doses by the IV or oral routes produced similar ^{14}C concentration curves, which further indicates the nearly complete absorption following oral doses. Urinary excretion of ^{14}C following oral administration of 100 mg/kg was much slower than when the same dose was introduced by the IV route; the accumulated percent excreted in the urine was 11/9 $\pm$ 5.4% and 32.0 $\pm$ 7.0% at 8 and 16 hours, respectively.

The petitioner is currently carrying out experiments to identify and characterize the majority of ^{14}C activity. Ether extraction of acidified urine samples from rats has resulted in the extraction of 96 to 98% of the ^{14}C activity in the urine. When TLC of these ether extracts is performed, most (at least 95%) of the extracted ^{14}C from two rats was located at the R_f of standard Dowco 233. TLC values for another rat treated IV with 100 mg/kg showed most of the extracted ^{14}C was located at the Dowco 233 position, but 10 to 20% was located in the position of 3,5-trichloro-2-pyridinol. These studies are not yet completed. NOTE: These experiments (the interim report) was reported in April, 1976.

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Dogs - Recovery and routes of excretion of ^{14}C. The overall recovery of ^{14}C administered by the IV route for 3 doses (3 dogs; 0.5, 5 or 20 mg/kg) averaged 94.7 $\pm$ 2.6%, and the average for 2 dogs dosed orally (0.5 or 20.0 mg/kg) was 85.3%. There was a significant dose-related response in the routes of excretion following IV administration. The percent of ^{14}C excreted in the urine was 95.1, 88.7 and 54.7% at the 0.5, 5 and 20 mg/kg dose levels; these values were accompanied by a simultaneous increase in fecal excretion of 0.2, 5 or 23%, respectively. The total quantity of ^{14}C excreted by both routes for dogs treated by the IV route was 95.3, 93.7 and 77.7% for dogs treated with 0.5, 5.0 or 20 mg/kg. A similar result for dogs treated orally with 0.5, or 20.0 mg/kg with a decrease in urinary ^{14}C activity and an increase in the quantity of ^{14}C in fecal excretion. The increase in urinary ^{14}C following oral administration was comparable to or greater than that following IV administration; which indicates extensive absorption of ^{14}C Dowco 233 following oral administration.

Tissue Concentration of ^{14}C - At the two lower IV dose levels (2 dogs - 0.5 or 5.0 mg/kg) the kidney was the only tissue examined that contained measurable quantities of ^{14}C. At 20 mg/kg all the tissues examined except brain contained measurable ^{14}C; the highest concentration was in the kidney (equivalent to 23 ug/g of tissue).

Oral administration at 0.5 or 20.0 mg/kg produced a similar pattern of ^{14}C.

Blood Plasma (dogs) - When 3 dogs were treated IV with 0.5, 5.0 or 20.0 mg/kg ^{14}C Dowco 233, graphic plots of plasma ^{14}C concentrations indicated dose-dependent clearance effects. ^{14}C plasma curves at 0.5 or 5.0 mg/kg show biphasic patterns; with half-lives of about 14 hours; ^{14}C decline at the highest dose level was monophasic with a half-life of about 96 hours.

^{14}C plasma concentration curves for two dogs treated orally with 0.5 or 20.0 mg/kg indicated rapid absorption; peak levels attained about 0.5 hours post-treatment. The average terminal half-life of each curve was about 14 hours following the low dose and 40 hours after the high dose.

Urinary ^{14}C - Initial urinary excretion rate of ^{14}C was about the same for dogs dosed orally or by IV at 0.5 mg/kg, while accumulation of ^{14}C in urine was slower at high dose levels. The cumulative appearance rate of ^{14}C in urine at an oral 20 mg/kg dose level was more rapid than for the same dose administered IV.

Identification of ^{14}C excreted material is not yet complete; however the petitioner states that work is in progress to characterize the ^{14}C urinary activity from dogs. Ether extraction of acidified urine samples from dogs treated IV with 0.5 or 5 mg/kg resulted in recovery of about 99% of the urinary ^{14}C; TLC of the ether extracts showed that almost all of the extracted ^{14}C was located at the R_f of standard Dowco 233. A similar extraction of urine from the dog that received 20 mg/kg IV resulted in recovery of 82% of the urinary ^{14}C; TLC showed that all of the ^{14}C activity was located at the position of standard Dowco 233. The petitioner states that additional studies of urinary Dowco 233 product(s) will be included in a final report.

Summary - Following single rat IV or oral doses ranging from 5 to 100 mg/kg, 92.1% of radioactivity was recovered from excretory routes; tentatively identified as parent Dowco 233. Overall recovery of radioactivity by excretory routes for dogs treated IV was 94.7%, and 85.3%, when administration was by the oral route. Final study report should contain studies intended to identify radioactive Dowco recovered from rat and dog excretory routes, and also indentification of radioactive compounds recovered from various tissues.

Supplementary Study (Study may be upgraded to Core-Minimum following submission of completed study).

7) Pharmacokinetic Profile of ^{14}C Dowco 233 following IV Administration in the Rhesus Monkey. Tester: Dow Chemical Co. Toxicology Research Laboratory, Health and Environmental Research, Midland, Michigan. August 19, 1976.

Test Material - ^{14}C Dowco 233

The tracer had a specific activity of 1.18 mCi/mmole (4.58 uCi/mg). The ^{14}C Dowco 233 was two dimensionally CO-chromatographed with standard unlabeled Dowco 233 (99.6% pure) in each of three TLC systems. Liquid scintillation counting of segments scraped from the TLC plates showed that at least 98% of the radioactivity was located at the position of standard Dowco 233.

A monkey was given an IV dose of 30 mg ^{14}C Dowco 233/kg. Blood plasma, urine and fecal samples were obtained over a period of 10 days following treatment. The same monkey was then given another dose (IV) of 30 mg ^{14}C Dowco 233/kg; blood, urine and fecal samples were collected for a period of 6 days. At the end of this time (16 days) the monkey was killed and various tissues and organs were removed for ^{14}C analysis. Table 1 presents dosing details:

TABLE I. ^{14}C-DOWCO 233 intravenous dosing solutions in 0.05M Sorensen's phosphate buffer, Ph 7.4.

	First Dose	Second Dose
Concentration of DOWCO 233	10.1 mg/ml	10.3 mg/ml
Solution radioactivity[1]	3.32×10^6 dpm/g (±3.1%)	3.45×10^6 dpm/g (±4.0%)
Specific activity of DOWCO 233	328 dpm/ug	334 dpm/ug
Administered radioactivity	56.2×10^6 dpm (25.3 uCi)	58.9×10^6 dpm (26.5 uCi)
Administered dose level	301 mg/kg	33.5 mg/kg

[1]Plus or minus standard deviation of three determinations.

Each dosing solution was administered through a cannula over a 15 minute period so that the solution was delivered at a rate of about 1 ml/minute; the specific input rate was approximately 2 mg/kg/min.

Sample Collection - For the first 72 hours following dosing, heparinized blood samples were obtained through an indwelling cannula. The monkey was transferred to a holding cage after 72 hours; succeeding doses and urine samples were obtained by veni-puncture at 0.25, 1, 2, 3, 4, 6, 8, 10, 12, 20, 24, 28, 32, 48, 53, 72, 96, 144, 191, 220 and 240 hours post-dosing.

Urine was collected through a plastic cup placed under the monkey's penis into a tared container immersed in a dry ice bath. Fecal samples were collected at 8 hour intervals from a plastic pan placed under the holding chair. All fecal samples were stored frozen until analyzed for ^{14}C.

The monkey was killed at experiment termination; tissues and organs were then collected for ^{14}C analysis. All tissues and organs were stored frozen until analyzed.

All $_{14}C$ determinations were carried out with a liquid scintillation system, and were corrected for background radiation and converted to dpm for subsequent calculations. Three sections weighing 100 to 150 mg of each tissue and organ were oxidized and counted. Samples of bone marrow, testes and eyes were analyzed for ^{14}C pulverizing tissue at liquid N_2 temperatures prior to scintillation counting.

Analysis of Urinary - ^{14}C - 2 ml portions of the 0-8 hour post-dosing urine sample were diluted to 5 ml with water, adjusted to pH 1, saturated with Nacl, extracted 3x with 5 ml amounts of ether. Additional 2 ml urine samples similarly treated were hydrolyzed prior to TLC. Ether was evaporated from combined extracts (per sample), the sampler was taken up in ETOH and subjected to TLC, using 3 different solvent systems. 8-16 hour urine samples (post-dosing) were similarly treated. Unlabeled standard Dowco 233 was Co-chromatographed with the urine samples.

Data Analysis - Blood plasma ^{14}C concentrations were converted to ug equivalent of ^{14}C Dowco 233 per ml of plasma based on specific activity of administered ^{14}C Dowco 233. Plasma concentration vs log weight time values were then fitted to a two-compartment linear pharmacokinetic model by a modified nonlinear parameter estimation route. A set of differential equations describing the two-compartment plasma model was combined with the pharmacokinetic parameter obtained above in order to simulate plasma concentrations arising from multiple doses of ^{14}C Dowco 233, using a computer.

Semi-log plots of the resulting data plots were then drawn. Renal clearance of ^{14}C activity was calculated by dividing the urinary excretion rate of ^{14}C 9as ug of ^{14}C Dowco 233) by the concentration of ^{14}C in plasma (as ug of ^{14}C Dowco 233 per ml).

Results:

Blood Plasma ^{14}C - The ^{14}C concentration in blood plasma following each dose of ^{14}C Dowco 233 showed the biphasic clearance typical of a two-compartment model system. The ^{14}C concentration data in blood plasma following the first dose were used to obtain the two-compartment pharmacokinetic parameter estimates shown in Table 2:

TABLE II

Pharmacokinetic parameters characterizing the two-compartment plasma model following a single intravenous dose of 30 mg ^{14}C-DOWCO 233 per kg in the rhesus monkey.

Parameter	Value ± Standard Deviation
Volume of distribution, V_1	127 ml/kg ± 5.6
Transfer rate constant, k_{12} (plasma - tissue)	0.117 hr^{-1} ± 0.004
Transfer rate constant, k^{21} (plasma tissue)	0.010 hr^{-1} ± 0.001
Elimination rate constant, k_{10}	0.103 hr^{-1} ± 0.005
Half-life, $t_{1/2}^{(a)}$	3.07 hr
Half-life, $t_{1/2}^{(b)}$	151 hr

The majority of the ^{14}C was cleared from plasma with a half-life (α phase) of 3.07 hours, and the remainder was cleared at a slower rate with a half-life (β phase) of 151 hours. The volume of distribution (V_1) was 127 ml/kg.

The concentration of ^{14}C in RBC was determined at 28, 48 and 90 hours following the second dose and in each case was found to be less than one-half of the concentration of ^{14}C in plasma.

Excretion of Urinary ^{14}C - A total of 69.4% of the ^{14}C administered with the first dose was recovered in the urine by 10 days post-dosing. A total pf 82.0% of the ^{14}C administered with the second dose was recovered in the urine by 6 days following the second dose. An average of 95.7% of the urinary ^{14}C was excreted within the first 24 hours following each dose. After the first 48 hours following each dose the urine excretion had fallen to an average of approximately 0.3% of the administered dose per day. At the end of the experiment the total ^{14}C recovered in the urine accounted for 75% of the total administered ^{14}C.

During the first 24 hours following dosing, the excretion rate of ^{14}C in the urine was nearly constant, averaging 24.1 $\pm$ 6.1% per 8 hour interval. For the 24 through 40 hour period, urinary ^{14}C was eliminated at an apparently first oder rate with an average half-life of 2.58 $\pm$ 0.22 hour.

The average renal clearance of ^{14}C for the 5 successive 8 hour intervals following each dose was 0.2, 0.9, 1.3, 0.2 and 0.1 ml/minute/kg, respectively. Renal clearance of ^{14}C during the later time periods was variable and averaged about 0.02 ml/minute/kg.

Identification of Urinary ^{14}C - An average of 99% and 93% of the ^{14}C in the urine was extracted into the ether phase from the non-hydrolyzed and hydrolyzed urine samples, respectively. TLC of these extracts showed that the non hydrolyzed urine samples contained approximately equal amounts of ^{14}C at the origin and at the location of the unlabeled Dowco 233; however, the acid-hydrolyzed urine samples contained ^{14}C labeled material only at the location of the Dowco 233. TLC of the ether extracts from the non-hydrolyzed 8 to 16 hour urine sample showed ^{14}C only at the origin; thus the conjugated form of urinary ^{14}C Dowco 233 was apparently stable in the acidic extraction conditions.

Tissue Concentrations of ^{14}C - Blood plasma contained the highest concentration of ^{14}C, equivalent to 8.6 ug Dowco 233 per g. The concentration of ^{14}C in the kidney and liver were equivalent to 1.7 and 0.8 ug/g, respectively. None of the tissues or organs contained a higher concentration of ^{14}C than did blood plasma. The ^{14}C contained in all the tissues and organs represented only 0.321% of the total done of ^{14}C.

Total Recovery of ^{14}C - The total quantity of ^{14}C recovered in feces throughout the experiment was 0.7% of the cumulative dose of ^{14}C was recovered in chair and cage washes. The total quantity of ^{14}C recovered in excrete (urine, feces, and cage washes) was 78.0% of the cumulative dose of ^{14}C. Since 0.32% of the cumulative dose was recovered in tissues and organs from the monkey carcass, the overall ^{14}C recover was 78.32% of the total administered ^{14}C.

Dogs administered ^{14}C Dowco 233 IV at 20 mg/kg showed a first order rate of clearance of ^{14}C from blood plasma with a half-life of 96 hours; which is much slower than the phase of ^{14}C plasma clearance observed in the monkey.

Urine is the major route for elimination of ^{14}C in the monkey, as it is in the rat and dog.

At 6 days following the second dose of ^{14}C Dowco 233 the total observed quantity of ^{14}C in the urine was 78.0%; thus, much of ^{14}C in the urine was unaccounted for. The petitioner states that a quantity of ^{14}C may have been eliminated by an undetermined route, perhaps as a volatile degretative product of the parent compound, or as expired CO_2. If $^{14}CO_2$ elimination did occur, the widespread distribution of low levels of ^{14}C in the monkey carcass may reflect the incorporation of C_1 units derived from ^{14}C Dowco 233 into the metabolic pool.

The rapid clearance of the majority of the ^{14}C from blood plasma, and the efficient renal excretion of ^{14}C, and the very low levels of ^{14}C detected in the carcass suggest that Dowco 233 should not accumulate to excessive levels in the monkey following repetitive doses of approximately 30 mg or less/kg.

Classification: Core-Minimum Data

8. Renal Function Studies with Dowco 233 in the Dog and Monkey. Sponsor: Dow Chemical Co. Tester: Institute of Comparative and Human Toxicology, Albany Medical College, Albany, N.Y., and International Center of Enviromental Safety, Albany Medical College, Holloman AFB, New Mexico. Dog Study begun 2/7/1977, Monkey Study begun 11/15/1977.

Test Material - Dowco 233 (technical) dissolved in Sorensen phosphate buffer, pH 7.0.

Four male rhesus monkey were given 5 mg/kg daily by gavage, seven days per week for 28 days.

Following this treatment, the dosage was increased to 20 mg/kg daily; the increased dosage was continued for 102 consecutive days.

Two Beagle dogs were treated by gavage with 5 mg/kg each for a total of 47 consecutive days.

Observations - Daily observations were made for signs of toxicity, changes in appearance or behavior and pharmacologic or other effects.

Body Weights - Individual body weights of the monkeys were measured prior to start of the test, and were measured on six occasions thereafter. The dogs were weighed at the beginning of daily dosing and after dosing stopped.

Clinical Chemistry Studies

creatinine - mg/dl
blood urea nitrogen (BUN) - mg/dl
glutamic oxalacetic transaminase (SGOT) - U/l
glutamic pyruvic transaminase (SGPT) - U/l
sodium (Na) - MEq/l
potassium (K) - Meq/l

Urinalysis Studies

specific gravity
pH
glucose
protein

Excretion of phenolsulfonthalein (PSP)

The rate of PSP excretion was measured in each monkey and dog before treatment with Dowco 233 was started, and was measured at various intervals thereafter. The animals were fasted overnight and a clinical test dose of PSP (6 mg) was given IV. Urine was then collected using a catheter over the following 20 minutes; the bladder was flushed with saline to insure complete collection of PSP. The amount of PSP excreted during the 20 minute interval was expressed as a percent of the original 6 mg dose. Usually, the PSP excretion studies were performed in the morning prior to the daily Dowco 233 dose. In one instance (after 85 days of dosing monkeys with 20 mg/kg), the Dowco 233 dose was infused IV, instead of given orally.

Inulin and para aminohippurate (PAH) Clearance

Clearance rate of inulin and PAH were determined in monkeys by means of a modification of the constant - infusion technique of Cole, et.al.

B.R. Cole, J. Giangiacomo, J.R. Inglefinger, and A.M. Robson.

Measurement of renal function without urine collection. N. Eng. J. Med. 287:1109-1114, 1972.

Inulin and PAH clearance were not measured in the day.

Results - Monkeys

Observations - The monkeys tolerated Dowco 233 well at both test doses; however, diarrhea or soft stools occurred occasionally during administration of Dowco 233 at 5 mg/kg. The frequency of these effects increased when the dose was raised to 20 mg/kg. Water consumption was not measured; it was observed that the monkeys showed an increased thirst and emptied their water container more rapidly and frequently than normal.

Body Weights -- The body weight increased slightly during the experiment.

Clinical Chemistry - The values of potassium concentration were slightly higher after Dowco 233 administration was begun than during the pre-treatment period; however, the tester states that the increased values were within the normal potassium limits for their monkey colony. The other serum values were also stated to be within the normal limits displayed for the tester's monkey colony.

Urinalysis - There were no observable effects on urinalysis from administration of Dowco 233.

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Excretion of PSP - presented in Table 1 below:

Excretion of phenolsulfonphalein (PSP) by monkeys receiving Dowco 233 at 5 mg/kg for 28 days and then at 20 mg/kg thereafter. The data are expressed as per cent of PSP excreted during 20 minuted following injection of a 6 mg dose.

	Animal Number			
Days of Dosing (at indicated dosage)	1807	1958	2075	2113
Baseline 1	-	18	30	22
Baseline 2	32	25	28	27
Baseline 3	33	28	34	25
5 mg/kg				
8	17	23	20	7[a]
24	23	56	51	28
20 mg/kg				
24	35	36	40	32
37	52	52	52	49
72	40	45	45	37
85[b]	28	30	30	28

[a]Data for animal #2113 invalid due to failure to inject all of the 6 mg dose of PSP.

[b]DOWCO 233 administered intravenously on this day simultaneously with PSP.

PSP values determined after 8 days treatement with 5 mg/kg were slightly reduced, and showed no reduction when repeated after 24 days of dosing.

PSP values were not reduced when the daily Dowco 233 dose was raised to 20 mg/kg and excretion rates were determined 4 additional times.

Clearance of Inulin and PAH

Dowco 233 did not have an effect on clearance of Inulin and PAH in rhesus monkeys.

Results - Beagle Dogs

Observations - The two dogs treated with 5 mg/kg of Dowco 233 for 47 days did not demonstrate any adverse treatment effects, other than soft stools on a few occasions. Both animals maintained their weight during the study. One dog maintained a constant 11.5 kg and the other dog increased from 12.0 to 12.3 kg.

Clinical Chemistry - The values for sodium and potassium were slightly higher after Dowco 233 dosing was initiated than during the control period, but remained within normal limits. Both dogs displayed elevated transaminases (SGOT, SGPT) during the control period and this limited the significance of their measurements. Toward the end of the study the transaminase values were in the normal range. The remaining measured clinical chemistry values were normal throughout the study.

Urinalysis - There was no apparent effect of Dowco 233 on urinalysis in Beagle dogs.

Excretion of PSP - The values measured after both 17 and 31 days of dosing with Dowco 233 indicate reduced rates of PSP excretion by the two dogs tested, as indicated in Table 2:

TABLE 2

Excretion of phenolsulphonphthalein (PSP) by Beagle dogs receiving DOWCO 233 at 5 mg/kg daily. The data are expressed as per cent of PSP excreted during 20 minutes following injection of a 6 mg dose.

	Animal Number	
Day of Dosing	D-1	D-2
Baseline 1	28	28
Baseline 2	24	29
17	12	18
31	<8	20

Conclusions :

1. Rhesus monkeys tolerated Dowco 233 well at daily doses up to 20 mg/kg; the only effects noted were some diarrhea and some occurrence of softened stools.

2. Dowco 233 at doses of up to 20 mg/kg daily doses does not have an effect on renal function in rhesus monkeys measured by PSP excretion or inulin and PAH clearance.

3. Daily administration of Dowco 233 at 5 mg/kg dose levels reduces the rate of PSP excretion in Beagle dogs.

4. A NOEL for impairment of renal function in dogs treated with Dowco 233 was not established; the highest dose level tested was 20 mg/kg.

Classificaation - Core-Minimum

9. Acute Oral LD_{50}, Male and Female Rats Administered Dowco 233 Pesticide. Sponsored by Dow Chemical. Performed by the Dow Toxicology Health and Environmental Sciences Research Laboratory, Midland, Michigan.

Test Material - 3,5,6-trichloro-2-pyridyloxy acetic acid, also known as triclopyr or Dowco 233. Identified as AGR 134832.

Following overnight fasting, five groups of Sprague-Dawley rats/sex/dose level were given 200, 400, 630, 800 or 1600 mg/kg/rat, as a 10% solution in corn oil by single dose gavage. Each rat was weighed just before treatment and weekly thereafter for a 14 day observation period. Rats were observed periodically for toxic signs. Survivors were subjected to gross pathological examination 2 weeks post-treatment. The acute oral median lethal dose was calculated by the moving average method of analysis (Thompson and Weil, 1952. Biometrics, Vol. 8, No. 1, pp. 51-54, as implemented in a computer program). The slope was calculated as $\log_{10} (LD_{84}/LD_{16})$.

Results - Toxic signs for the 200 and 400 mg/kg groups ranged from none to slight legarthy. Some females in the 630 mg/kg group exhibited exudate around the nose, piloerection, physical weakness.

Dose related similar toxic signs were observed for the 800 and 1600 mg/kg groups. All surviving rats gained weight during the 2-week post-treatment period. A male rat survivor of the 800 mg/kg group had a decreased thymus upon gross pathological examination - which may have been stress related. All other gross lesions were too sporadic to be considered treatment-related.

Mortality

Dose mg/kg	Dead/Treated Males	Females
200	0/6	0/6
400	0/6	0/6
630	1/6	3/6
800	5/6	5/6
1600	6/6	6/6

Acute oral LD_{50}, male rats = 729 mg/kg (515-1127 mg/kg, 95% C.L.), slope = 6.92.

Acute oral LD_{50}, female rats = 630 mg/kg, (450-829 mg/kg, 95% C.L.), slope = 5.23.

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Toxicity Category III
Classification - Core-Minimum Data

10. Subchronic Dietary Feeding Study in Beagle Dogs
Sponsor: Dow Chemical Co. Performed by the Dow Toxicology Research Laboratory, Health and Environmental Research, Midland, Michigan. July 21, 1976.

Test Material: 3,5,6-trichloro-2-pyridyloxy acetic acid (Dowco 233).

A preliminary dietary acceptance study was conducted using two dogs administered 100 mg/kg/day and 30 mg/kg/day, respectively. The dogs refused to eat the treated feed for 3 days, and were then offered the test chemical in feed at 10 and 20 mg/kg/day for 10 days without adverse effects. Based on these results, dietary levels of 0, 5, 10 and 20 mg/kg/day was administered to dogs in a 228 day study:

Treatment Group	Dose mg/kg/day	Number of Dogs Males	Females
1	0	4	4
2	20	4	4
3	10	4	4
4	5	4	4

During a 3 month acclimatization period, baseline body weight and food consumption data were collected. Dogs were 14 months old at start of the study. Water and control feed and treated feed were available ad libitum.

The concentration of test chemical in diets was adjusted weekly according to the rate of food consumption and dog body weights to maintain the designated dosage. Prior to starting the study, the stability of the test chemical was established by periodically analyzing a diet containing 1% test material.

Dogs were observed at least once each day for changes in appearance and behavior. A veterinarian examined each dog every day during the pre-treat period and during the study.

Opthalmic examinations were conducted by slit-lamp opthalmoscopy during the pre-test period at 173 days; and at 224 days. Body weights were measured weekly. Food consumption for each pen of 4 dogs were measured twice weekly throughout the study. The average amount of food consumed/dog/day was calculated. Terminal body weights were recorded after dogs had been fasted overnight.

Clinical Studies

Hematology - PCV (packed cell volume), RBC, Hgb (hemoglobin concentration), WBC, WBC differential measurements were made on all dogs prior to beginning the study and at 172 and 225 days. Reticulocyte counts were made after 225 days.

Urinalysis - Urine samples were collected from all dogs prior to starting the study and at 177, 180 and 224 days. A urine sample was aspirated at necropsy. Also, at 181 days a urine sample was collected by catheterization following a 24 hour water withholding period to evaluate urine concentration ability of the kidneys. The sp. gr., pH, sugar, protein, ketones, occult blood, bilirubin, and microscopic examination of urine sediment tests were made.

Clinical Chemistry - BUN (blood urea nitrogen), AP (serum alkaline phosphatase activity), SGPT and SGOT were determined on all dogs prior to initiation of the study, and at 76, 167, 194 days, and at necropsy. At 176 days a serum sample was also collected to evaluate total and direct and indirect reacting bilirubin levels. At 201 days, serum samples were collected and sent to Bio-Science Laboratories for determination of SGPT, OCT (ornithine carbamyl transferase) activity, and gamma-glutamyl transpeptidase (GGT). Also at 201 days, a sulfobromothalein (BSP) clearance rate was determined to evaluate liver function. At necropsy, a serum sample was collected to determine OCT. Renal function was further tested by measuring phenolsulfonthalein (PSP) excretion at 224 days.

Necropsy and Pathology - At termination (228 days M, and 229 days F), dogs were fasted overnight, weighed, and exsanguinated (killed and bled). Gross pathologic examination were made on all dogs. The brain, heart, liver, kidneys, adrenals, and testes were removed and weighed. Representative portions of the following tissues were preserved and prepared by routine histological procedures, stained, and examined microscopically for histopathologic alterations: heart, liver, kidneys, testes, brain, pituitary gland, thyroid and parathyroid glands, trachea, esophagus, tongue, lungs, aorta, stomach, salivary glands, spleen, skin and mammary tissue, gall bladder, pancreas, small intestine, colon, mesenteric and mediastinal lymph nodes, thymus, tonsil, urinary bladder, urethra, epididymis, prostate, ovaries, uterus, skeletal muscle, sciatic nerve, spinal chord, adrenals, sternum, and sternal bone marrow. Eyes were preserved in Zenker's fixative. Liver sections from all treated and control animals were stained for Gomori's iron reaction to characterize pigmented material recognized in H&E stained sections.

Statistical Analysis - Hematology, clinical chemistry, food consumption, body weight, organ weight, and organ to body weight ratio data were evaluated by analysis of variance and Dunnett's test; $p < 0.05$. Due to limited sample size and individual variability in clinical chemistry determinations of SGPT, AP, and OCT, comparison of the mean control values and treated groups did not detect differences which appeared to be treatment related. Therefore, clinical chemistry values for GGP, AP, observations more than 2 standard deviations higher than the mean for several groups of control dogs from recent studies in the Dow Laboratory were considered significant.

Results

Appearance, Behavior and Body Weight

Female dogs showed a statistically significant loss of body weight at all three dose levels., and the top and middle dose females showed slight hair thinning. Male dog body weights did not show any statistically significant body weight loss, individuals in the top and middle dose groups frequently showed decreased body weights, compared to pre-treatment weights. One female in the top dose group displayed some limb stiffness, three dogs developed a focal dermatitis; all responded to topical treatment.

Mean Daily Food Consumption

Female dogs at all dose levels consumed statistically significantly less total amounts of food.

Mean non-fasted pre terminal and fasted terminal body weights of female dogs at all dose levels are shown below:

Dose mg/kg/day	non-fasted pre terminal weight (kg)	Fasted terminal weight (kg)
0	11.1	10.6
± S.D.	± 1.1	± 0.9
20	8.5*	8.3*
± S.D.	± 0.1	± 0.3
10	9.4*	8.7*
± S.D.	± 0.2	± 0.3
5	9.0*	8.4*
± S.D.	± 0.6	± 0.5

*Statistically different from controls by analysis of variance and Dunnett's test, $p < 0.05$.

A statistically significant decrease in the mean total food consumption throughout the study was seen in female dogs at all treatment levels. However, when the decrease in food uptake by the control females during the test interval is compared to the pretest values, the food consumption by females at the two lower dose levels was not dissimilar from that of the control females. Mean food consumption of male dogs throughout the study was not significantly less than controls; however, males on treatment diets consumed less food during the study compared to pre-treatment values.

Clinical Studies - Hematology - Low dose male dogs showed an elevated pre-test WBC count, which was further elevated at 172 days. Top dose females showed decreased PCV, RBC counts and Hgb concentration at 172 days, while top dose males showed a decreased PCV and Hgb. Pre-terminal top dose females PCV, RBC counts and Hgb concentration showed statistically significant decreases, while top dose males showed decreased Hgb.

Urinalysis - There was no evidence of a clear-cut dose response on urinalysis that could be treatment related. An increase in urine specific gravity following withholding water was believed to indicate normal kidney ability to concentrate urine.

Clinical Chemistry - There were no alterations in BUN values considered to be treatment related. Increased SGPT values were observed in top dose male dogs and in females at all dose levels at 167 and 176 days. Elevated SGPT values in top dose females were observed at 194 and 201 days and at necropsies.

Increased AP values were found in top dose females at 76, 167 and 176 days at necropsy. Elevated SGOT values in top dose females at 167 and 176 days and at necropsy were observed. In top dose females, SGOT values were elevated at 76, 167, 176 and 194 days. BSP values determined at 201 days indicated an increased retention of the dye in both males and females of the middle and top dose levels. SGPT values for lowest dose females were only slightly increased at 167 and 176 days; however, SGPT values of individual males and females from all dose levels were markedly increased at various times.

Phenolsulfonthalein (PSP) excretion values decreased at 224 days for both male and female dogs at all treatment levels, compared to controls.

Opthalmic examination by slit-lamp did not reveal any treatment related changes.

Pathology - Body and organ weight alterations considered treatment related included: decreased terminal body weights of female dogs at all dose levels; increased relative liver weights in males receiving the top and middle dose and females receiving the top dose level; increased relative kidney weights of females receiving the top and middle dose levels. The petitioner stated that a statistical increase in relative heart weights of females at all dose levels may have been related to the significant decrease in overall body weight. A significant decrease in absolute weight of adrenals of females from the top and low dose levels was considered of unknown origin. A variety of pathologic alterations were present in both control and treated dogs that were considered unrelated to the test material. Apparently treatment related changes were: a decrease in the amount of adipose tissue in the top dose female dogs; microscopic changes in the liver and kidney of treated males and females at all dose levels. Liver changes included a decreased eosinophilic staining of hepatocytes with focal areas of coagulation and necrosis and proliferation of the reticuloendothelial cells lining the sinusoids.

Treatment related microscopic changes in the kidney were found in dogs at all dose levels, including cloudy swelling characterized by a decrease in eosinophilic staining of epithelial cells with increased amounts of granular pigmented material.

Thus, dogs receiving Dowco 233 in the diet exhibited toxicological symptoms at all dose levels. The kidney and liver consistently showed alterations in the parameters tested. No NOEL was established in this study; the NOEL is < 5 mg/kg.

Classification - Core-Minimum Data

Supplemental Study of PSP Excretion (no additional information)

Based on the chemical structure of Dowco 233 and the results of the 6 month study the existance of a competitive mechanism of renal excretion for the test material and the dye appeared to be the most probable explanation for the observed alteration in PSP excretion.

Four male dogs were tested as follows: 2 dogs were administered 2 mg/kg/day in divided doses in gelatin capsules twice daily for 16 days, and then administered 2 mg/kg/day in the diet for an additional 12 days. Following this 38 day Dowco 233 treatment at 2 mg/kg/day, the dosage in the diet of the two dogs was increased to 20 mg/kg/day for an additional 18 days.

Two male dogs were similarly treated with 0.5 mg/kg/day for 38 days, followed by a dietary increase of Dowco 233 to 5mg/kg/day for an additional 18 days.

0.5 mg/kg/day administered by gelatin capsule, or incorporated in the diet did not alter the PSP excretion rate, while 5 or 20 mg/kg/day produced marked decrease in PSP excretion rate. The results suggested a competitive mechanism of renal excretion for the test material or its metabolite(s) and the PSP dye.

NOTE: A 0.5 mg/kg/day NOEL for PSP excretion was determined in this study (dogs fed Dowco 233).

Classification - Supplementary Data (More dogs should have been employed in this experiment, and PSP excretion measurements should have been made more frequently).

11. Supplemental Subchronic Dietary Feeding Study in Beagle Dogs (Dowco 233). Performed by the Dow Toxicology Research Laboratory, Health and Environmental Research, Midland, Michigan. July 21, 1976.

Test Material - 3,5,6-trichloro-2-pyridinyloxy acetic acid (Dowco 233).

The previously reported Dow subchronic dietary feeding study in dogs at dose levels of 0, 5, 10, or 20 mg/kg/day indicated treatment related decreased food consumption and body weight gain, altered organ weights of liver and kidneys, and changes in clinical chemistry tests indicating altered liver and kidney function with minimal microscopic pathologic changes observed during the 6 month study.

The present study was designed to evaluate certain parameters in the dog that were affected by treatment with higher doses of test material in the previous study.

Thirty-four Beagle dogs were acclimated during which time baseline body weight and food consumption data were obtained. The dogs were grouped and tested as follows:

Treatment Group	Dose mg/kg/day	Number of Dogs	
		Male	Females
1	0	5	5
2	0.1	4	4
3	0.5	4	4
4	2.5	4	4

Water and feed supplying test material were available ad libitum. Male dogs were on test 183 days and female dogs for 184 days.

The test diets were prepared weekly throughout the study with test material concentration being adjusted according to the rate of food consumption and body weight changes. A 1% pre-mix of Dowco 233 with feed was used to prepare the final dietary concentration. Previous tests showed that test material concentration did not change over a two week period. Aliquots of pre-mix and test diets were saved at monthly intervals for possible analysis.

Clinical Observations - Dogs were frequently observed for clinical signs of toxicity or changes in appearance or behavior. Body weights were recorded weekly during the first month of the study and every two weeks thereafter. Food consumption per pen of dogs was recorded twice each week throughout the study. The average amount of food consumed per dog per day was calculated.

Hematology - PCV, RBC, Hgb concentration, and WBC differential counts were made on all dogs on days 14, 30, 85 and 174 of the study.

Urinalysis - Catheterized urine samples from all male dogs were collected on days 8, 35, 89 and 168 of the study. Specific gravity, pH, sugar, protein, ketones, occult blood, bilirubin, and microscopic examination of the sediment were measured or recorded.

Clinical Chemistry - Dogs were fasted overnight and blood samples collected for BUN, AP, SGPT and SGOT. These determinations on all dogs were made on days 14, 30, 61, 84 and 174 of the study. The percent of sulfobromothalein (BSP) retention was determined on all dogs on days 13, 34, 92 and 176 of the study.

Renal function was indicated by determining the rate of phenolsulfonthalein (PSP) excretion from male dogs on 8, 35, 89 and 168 days, and for female dogs on 7, 36, 90, and 169 days of the study.

Opthalmologic Examination - The slit-lamp and opthalmoscope were used for opthalmic examination of all dogs on 21 and 177 days of the study. At necropsy, eyes and adjunct structures were examined for gross pathologic changes; eyes were removed and fixed in Zenker's solution. Eyes from dogs on the control and top dose level were prepared by histologic procedures for evaluation. Eyes from lower dose dogs were examined microscopically only if treatment-related changes were noted at the top dose level.

Pathology - At study termination; 183 days for male dogs, or 184 days for females dogs, animals were fasted overnight, exsanguinated, and gross pathological examinations were conducted on all dogs. The brain, heart, liver, kidneys, and testes were removed. Representative portions of the following tissues were removed and preserved in 10% buffered formalin: adrenals, aorta, thoracic bone (rib), sternal bone marrow, brain cerebral cortex, brain stem cerebellum, cecum, colon, male epididymes, esophagues, gall bladder, heart, kidneys, liver, lungs, lymph nodes (cervical, mediastinal, mesenteric), adipose tissue, skeletal muscle, parathyroid glands, pancreas, peripheral nerve (sciatic), pituitary gland, duodenum, jejunum, ileum, spinal chord, spleen, skin, mammary tissue, stomach, ovaries, thymus, tonsil, trachea, uterus and urinary bladder, as well as any tissue which appeared abnormal. In general, the above tissues from control and top dose level dogs were prepared by routine histological procedures,

stained with H&E, and were evaluated by light microscopy for treatment related changes. Selected tissues from middle and lower dose dogs were prepared and examined based upon the findings in the top dose dogs. Liver changes in iron content were detected by staining with Prussian Blue.

Statistical Analysis - Statistical evaluation of food consumption, body weights, organ weights, organ to body weight ratios, hematology, urine specific gravity and pH, and clinical chemistry data were made using an analysis of variance and Dunnett's test at a significance of $p < 0.05$.

Results

Clinical Observations - No treatment related changes in appearance or behavior were observed throughout the study.

Body Weights - No statistically significant changes in male or female dog body weights were detected at any dosage levels.

Mean Daily Food Consumption - The mean daily food consumption for treated male dogs was statistically significantly decreased at all dose levels tested; 0.1, 0.5, or 2.5 mg/kg/day, while female dogs did not show reduced food consumption when tested at these dose levels.

However, the body weights of male dogs were not altered during the course of treatment. An examination of tabular weekly mean food consumption for male dogs indicated that variation occurred and no consistent patterns related to treatment were apparent; it was believed that apparent reductions in mean daily food consumption probably reflect differences due to food wastage by control dogs, or could possibly be due to housing 5 control dogs in one cage, while 4 test animals were housed per cage. Thus, no toxicological significance was attributed to the statistically significant decrease in mean daily food consumption for treated male dogs.

Hematology- The only statistically significant differences in hematology were for male and female dogs receiving 2.5 mg/kg/day of Dowco 233, detected at 174 days of test. At this time there was a statistically significant increase in PCV and Hgb concentration for both sexes. In addition, in females only at this dose level the RBC count was significantly increased; however, all of these alterations were within the normal range of values as determined at the Dow Tox. Laboratory for the strain of dogs used.

Urinalysis - There was a statistically significant increase in urine pH observed in dogs that received 0.1 mg/kg/dy of test material for 35 days; no urine pH change was observed at any other dose, or at subsequent urinalysis. Also, the pH values that were significantly increased are comparable to those of individual dogs measured at the pre-exposure evaluation. Thus, there were no changes in urinalysis parameters tested which were considered treatment related.

Clinical Chemistry - The BUN was statistically significantly increased in male dogs that received 2.5 mg/kg/day of test material when evaluated at 124 days. This value was not observed at any other time and is within the normal range. The serum AP was significantly increased in females ingesting 2.5 mg/kg/day of test material at 61 days. There were no other statistically significant AP value alterations throughout the study; the mean AP activity for this group of dogs at 61 days was less than at 30 days, thus the one instance of AP elevation was not considered treatment related. No change in SGPT occurred. The SGOT activity was significantly increased in both male and female dogs that received 2.5 mg/kg/day of test material at 84 days. These increased SGOT activity values were within the normal range and were not considered treatment related. The SGOT values of both male and female control animals at 174 days were greater than the apparent increases noted for the 2.5 mg/kg/day test animals at 84 days.

Liver function tests with sulfobromopthalein indicated no differences in treated compared to control animals.

Renal function reflected by phenolsulfonthalein (PSP) excretion was determined prior to and repeatedly during the study. A statistically decreased rate of Dowco 233 excretion was observed in females after 90 days and in males after 168 days of ingesting the top dose of test material (2.5 mg/kg/day). No other alterations in PSP excretion were observed at other dose levels. These decreased rates of PSP excretion were considered treatment related.

Opthalmologic Examination - No treatment related ocular changes were revealed by slit-lamp and opthalmoscope.

Pathology - The mean absolute and relative kidney weights of male dogs ingesting 2.5 mg/kg/day of test material were statistically significantly reduced, which may be related to an impaired renal function determined by reduced PSP excretion reported above. Kidney weight changes were not observed at any other dosage levels for male dogs, nor in any of the females. Therefore, the present apparent kidney weight changes were not considered treatment related. The brain, heart, liver and testicular weights of male or female dogs did not show statistically significant alterations at any dose level.

There were no gross or microscopic pathologic changes in treated male or female dogs at any dose level. Kidneys of male dogs which showed absolute and relative decreased weight did not exhibit any microscopic alterations.

Prussian Blue staining of liver sections did not show a difference between treated and control dogs.

Summary - The only statistically significant alteration considered treatment related in the 6 month dog study was a decreased PSP excretion rate in dogs ingesting 2.5 mg/kg/day of test material; PSP excretion at the next lowest dose level (0.5 mg/kg/day) was unaffected.

A NOEL in this study was determined to be 0.5 mg/kg/day. PSP excretion rate, absolute and relative kidney weights were statistically significantly reduced, while SGOT values were statistically significantly increased at 2.5 mg/kg/day.

Classification - Core-Minimum Data

12. 28-Day Nasogastric Intubation Test in Rhesus Monkeys Using Dowco 233. Performed by the Dow Chemical Co. Department of Pathology and Toxicology, Indianapolis, Indiana. September 8, 1976.

Test Material - Dowco 233 (3,5,6-trichloro-2-pyridyloxy acetic acid). The test chemical was provided by the Dow Ag-Organics Dept., Midland, Michigan.

Dowco 233 was suspended daily in a 1% aqueous solution of hydroxypropyl-methyl cellulose NF 90 HG 400 cos (METHOCEL).

Four groups of 2 female monkeys each were placed on test:

Group	mg/kg/day
1	Vehicle
2	10
3	20
4	30

The test was conducted principally to determine any effects of Dowco 233 on the urinary system of the rhesus monkey, although other parameters were also investigated. Female monkeys were used because they could be catheterized for urine samples much more easily; reducing the possibility of irritation to the urinary tract.

Individual doses were calculated on the basis of individual body weights obtained weekly. Controls were dosed with vehicle equal to the largest volume given to any monkey during each week of test. The monkeys were treated daily by nasogastric intubation for a period of 28 days.

Clinical Observations -Physical condition and behavior observations were recorded daily. Food consumption was recorded daily and individual body weights were recorded weekly. A physical examination (heart rate, respiration rate, body temperature, eye examination and attitude) was conducted before the test began, and during the last week of the test. Indirect and slit-lamp opthalmologic examination were made prior to start of the test and during the final week of study.

Clinical Laboratory Studies - The following determinations were made on days 7 and 21 of the test:

hematocrit (Hct)
hemoglobin (Hgb)
total erythrocyte (RBC)
total leukocyte (WBC)
mean corpuscular volume (MCV)
mean corpuscular hemoglobin (MCH)
mean corpuscular hemoglobin concentration (MCHC)
reticulocyte count (Retic)
differential WBC count

Clinical Chemistry - Determinations were made on days 7 and 21 of the test:

blood urea nitrogen (BUN)
serum glutamic oxaloacetic transaminase (SGOT)
serum glutamic pyruvic transaminase (SGPT)
alkaline phosphatase (AP)
total protein (TP)
total bilirubin (TBR)

Urinalysis - Urine was obtained from each monkey on days 6 and 22 for the following: pH; specific gravity; color; turbidity; bilirubin, blood cells, protein, sugar, ketones and microscopic examination of sediment.

Evaluation of Renal Function - Phenolsulfonthalein (PSP) test. This test is used to measure the secretory capacity of renal tubules following the intravenous administration of PSP. Secretory capacity measurements were made on days 1, 3, 10 and 22. Each monkey was given 50 ml tap water/kg body weight via nasogastric intubation approximately 30 minutes before start of the test; then 1 ml (6 mg/ml) of PSP solution was injected IV. Approximately 12 and 27 minutes later the monkey was catheterized for withdrawal of urine at 15 and 30 minutes after PSP injection.

Urine Concentration - This test was conducted in order to determine the ability of the kidney to concentrate urine under conditions of water deprivation. The test is based on the specific gravity of urine obtained in the morning following withdrawal of drinking water. Monkeys were deprived of water at 4:00 P.M. on days prior to the concentration test. At 6:00 A.M. the following morning, two urine samples were collected from each monkey; water was provided again after the second urine sample was obtained. This test was conducted on days 5 and 24.

Post Mortem Studies - A thorough gross examination was made and representative tissues selected and fixed in 10% buffered formalin (eyes were fixed in cold 1% glutaraldehyde solution +1% buffered formalin for histologic evaluation).

Microscopic Studies - Sections of the following tissues were prepared for examination:

skin	sternbrae (bone marrow)
eyes	mammary gland
mandibular lymph node	liver
salivary gland	gall bladder
lung	kidney
heart	adrenals
trachea	pancreas
thymus	spleen
esophagus	colon
thyroid/parathyroid	spinal chord
ovaries/uterus	skeletal muscle
brain (3 levels)	sciatic nerve
pituitary	stomach
urinary bladder	small intestine (3 segments)
large intestine	

Electron microscopic examination was conducted on hepatic and renal tissue only.

Organ Weights

Weights for brain, pituitary, liver, kidneys, thyroids, adrenals, ovaries-uterus, spleen and heart were recorded. Statistical evaluation was not made, due to the small sample size.

Ancillary Evaluations - The following were collected for possible chemical analysis: whole blood; serum; liver, kidney and 24 hour urine output.

Analysis of Data - Due to the small sample size and great variability in compiled data, statistical analyses were not made, since the results would probably not be meaningful.

Results

I. Food Consumption and Body Weight

Individual body weights of female monkeys treated mice nasogastric intubation with Dowco 233 (kg).

TABLE I

Group Dose Animal No.	-7	0	7	14	21	28
1. Control						
566	4.50	4.25	4.35	4.35	4.35	4.35
544	4.90	4.50	4.65	4.75	4.85	4.70
2. 10 mg/kg/day						
378	4.40	4.30	4.35	4.50	4.55	4.45
490	4.95	4.50	4.40	4.50	4.35	4.30
3. 20 mg/kg/day						
488	4.15	3.90	3.80	3.90	3.90	3.85
556	4.95	4.50	4.55	4.85	4.80	4.45
4. 30 mg/kg/day						
484	4.50	4.25	4.35	4.50	4.55	4.45
388	4.30	3.25	4.35	4.40	4.30	4.55

The results shown in Table I indicate some variability in body weights for control as well as treated monkeys. This type of variation is common and not considered biologically siginificant.

There was no apparent effect on appetite, as shown by slight variation in food consumption during the test, also no consistent relationship between body weight fluctuations and observed variations in food consumption.

II. Clinical Observations - No significant effect on heart rate, respiratory rate and body temperature of monkeys on days 1 and 23 were noted. One monkey in the 20 mg/kg/day, and one monkey in the 30 mg/kg/day groups showed mild loss of appetite on the 3rd day of treatment, but regained appetite on the following day. Behavior and attitude of test and control animals were normal throughout the test.

Opthalmoscopic examinations did not reveal ocular alterations.

III. Clinical Laboratory Studies

a. Results of hematologic studies for monkeys after 21 days of treatment with Dowco 233 did not show significant changes in test parameters. One monkey (30 mg/kg/day) bled on day 7 did have a high glucose level; the WBC count for this monkey was elevated when measured on day 0, the WBC count had returned to normal range by day 21.

b. Clinical Chemistry Studies - Results of clinical chemistry determinations after 21 days of treatment did not reveal significant changes in the parameters tested.

c. Urinalysis - Routine urinalysis examinations on days 1, 6 and 22 did not reveal abnormalities.

IV. PSP Test Phenosulfonthalein) - A total PSP excretion rate of 40% or higher is considered desirable in evaluation of PSP results in man (renal function). In general, the control and test monkeys showed very good ability to excrete PSP at that rate. Slight variability in excretion rate appeared to be influenced not only by the hour of the day that the test was conducted, but also by the state of individual hydration even though all monkeys were given water intragastrically 30 minutes before the test. Dowco 233 treatment did not result in inhibition of PSP excretion on any of the test days.

V. Urine Concentration - Under the conditions of the test, Dowco 233 did not affect the ability of the kidney to concentrate urine.

VI. Post Mortem Examination

No attempt to statistically analyze organ weights was attempted due to the limited sample size. Variability in gonad weights was attributed to differences in age of the monkeys. A broad range in weights of the thyroid glands did not indicate treatment effects; microscopic examination of each organ weighed showed no evidence of treatment chemical effects.

Pathology - All monkeys were in good external condition at test termination. No treatment related gross alterations were found in any of the organs. Focal pulmonary alterations due to lung mite infestation in both control and treated monkeys were the most frequently observed gross and microscopic observations.

Histopathological studies confirmed the presence of pulmonary ascariasis in all monkeys. No alterations related to treatment with Dowco 233 were found. Electron microphotographs of renal tissue from high dosage monkeys showed there was no evidence of chemical effect on the ultrastructure of the kidney.

No toxic effects were detected in female rhesus monkeys orally dosed with 10, 20 or 30 mg/kg/day for 28 days.

Classification - Core-Minimum Data

13. 2-Year Oncogenic Evaluation of Dowco 233 (AGR 134832) in the Mouse. Performed by the Department of Toxicology, Dow Chemical Co., April 11, 1979. Project#T-637. Lab. Report and Code No. NBX-148.

Test Material - Dowco 233 (AGR 134832) (3,5,6-trichloro-2-pyridyloxy acetic acid).

Dietary Preparation - A 1% pre-mix of test material in Lab Blox ground meal was prepared and blended in a twin shell blender with more feed to obtain the dietary concentration of each treatment group. Fresh mixes were prepared every 4 weeks. Dietary mixes were stored at 4.4°C. Aliquots of all monthly dietary mixes were analyzed to establish intended doses.

Animals - CDF_1/COX mice were used. Before use, mice were approximately 28 days of age, and free of all observable disease. Starting body weights ranged from 20.7 to 22.1 g for males and 17.5 to 18.5 g for females. 5 mice per cage were housed in an air conditioned room with controlled temperature and humidity.

Study Design - Four hundred mice randomly assigned were distributed as follows:

Group	Number of Animals		Dowco 233, ppm (%) in diet
	Males	Females	
1	50	50	0 (control)
2	50	50	24 (0.0024)
3	50	50	80 (0.0080)
4	50	50	240 (0.0240)

Dietary treatment was continued throughout the study. The primary parameters evaluated were survival, body weights and clinical/pathological observations with emphasis on neoplasm incidence.

Clinical and Pathological Observations

Body weights were recorded every 14 days during the first 12 weeks of the test and monthly thereafter. Body weight represents the mean body weight per cage of mice. Food consumption was recorded monthly for 10% of the mice per group and sex over a 4 day period.

All mice were inspected at least 2x daily, seven days per week for clinical signs and mortality, and the observations generally were combined and recorded daily. The general physical condition of the animals were noted and recorded. Moribund animals were killed and necropsied. Animals were killed with CO_2, bled and immediately exsanguinated and necropsied.

At necropsy, gross findings for each mouse were recorded, tissues were collected and preserved in 10% buffered formalin. The following tissues were generally collected: gross lesions; tissue masses or suspected tumors; mandibular lymph nodes; pituitary; spinal chord and vertebrae which contained bone marrow; salivary glands; thyroid/parathyroid; thymus; lung; brain; eye; esophagus; stomach; small intestine; colon; liver/gall bladder; kidney; spleen; adrenal; skeletal muscle; pancreas; urinary bladder; uterus/ovaries; testes; prostate; epididymis; mammary gland; lacrimal gland; peripheral nerve; accessory sex gland and skin. The tissues were prepared, sectioned, stained with H&E for light microscope examination.

Data Recording and Statistical Analysis - Data were recorded in an automatic data processing system (body weights and feed consumption), and all other data in notebooks. Data elements included experimental design, animals, analysis of dietary mixtures, clinical observations, survival, body weights, feed consumption and individual pathologic results.

Body weights were analyzed by a one-way analysis of variance and followed by multiple comparison between control group means and other dose groups means using Dunnett's procedure. Homogeniety of group variances were tested by the Bartlett test. A non-parametric test of the two group means based on the Wilcoxon rank sum was also applied. In both cases, two-tailed and $p = 0.05$ values were the basis of significance.

Survival Analysis - Probabilities of survival were estimated by the product limit procedure of Kaplan and Meler. (Non-Parametric Estimation from Incomplete Observations. J. Amer. Statis. Assoc. 53:457-481, 1958). Statistical tests of differences in survival between control and treated groups were compared using the Fisher Exact Probability Test.

Tumor Analysis - The number of animals with tumors were analyzed as a percentage of the number of animals examined histologically. Analyses of the incidence of tumors were made with the Fisher Exact Probability Test to compare the controls to each treated group and by Tarone's method for testing a positive linear dose-related trend.

In all analyses of survival and tumor data, one-tailed and 5% significance levels were examined. Both survival and tumor analyses were made using the computer program developed at the NCI.

Results

Body Weights and Clinical Signs

Body weight gains for male and female mice were not inhibited by the test material; most treated mice showed greater weight gains than matched controls.

The appearance and behavior of treated and control mice were generally comparable throughout the test. Focal alopecia and/or dermatitis and small palpable nodules in the perianal area were seen in increasing numbers of male mice as the test progressed. These lesions were associated primarily with fighting between cage mates. The first palpable mass for a control male was recorded on day 217 and for a control female on day 533. The first palpable mass for a treated male was recorded on day 248 and for a treated female on day 368. The first death (day 111) was a control male with an obstructive uropathy.

Survival - The survival at 12, and 18 months, and at termination is shown below:

	Month	1(0)	2(24)	3(80)	4(240)
Males	1	50	50	50	50
	12	36	42	39	40
	18	26	25	21	42
	24	19	14	15	13
Females	1	50	50	50	50
	12	50	49	50	50
	18	43	43	45	50
	24	32	25	26	38

The higher incidence of death shown by males was related to the high incidence of obstructive uropathy, a syndrome which was encountered more frequently in groups 3 and 4:

Final Surival Rate (%), Male Mouse

Group	1	2	3	4
(dose, ppm/in diet)	0	24	80	240
	34	38	28	26

Statistically, there were no differences in male survival during the test.

The development of an obstructive uropathy resulted in male deaths in all groups and as high dose male survival approached the 10% level, all living males were submitted for necropsy on test days 681 and 682. This condition, which is considered to be under genetic control, occurred without relation to other pathologic findings, such as neoplasms. Females sustained a low mortality rate during the study and were terminated on days 728 and 729.

The development of male obstructive uropathy has been reported to be a genetic-related condition and is not treatment related. A similar condition, manifested as cystolithiasis, hemorraghic occlusion of the urethral sinus or suppurative vesicourethritis, has been reported by Sokoloff and Barile[1] and Babcock and Southan[2].

[1]Sokoloff, M.D. and Michael F. Barile. Obstructive Genitourinary Disease in Male STR/IN Mice. Amer. J. Path. 41:233-246(1962).

[2]Babcock, Virginia I. and Chester M. Southan. Obstructive Uropathy in Laboratory Mice. Proc. Soc. Exptl. Biol. Med. 120:580-581(1965).

Pathology - Non-tumor related pathology included: a relatively high incidence of systemic infection in female mice fed 24, 80 or 240 ppm of test chemical; 13/50, 13/50 and 12/50, respectively. Also eleven of 50 males fed 80 ppm test material showed fatty alteration at the histological level, and 9 of 50 control male mice showed focal inflammation of the skin upon histological examination.

A variety of tumors occurred in the control and treated mice. Malignant lymphoma was the most frequently encountered neoplasm and its incidence was higher in the untreated control mice. Each type of neoplasm was noted in test chemical diets; however, inspection of the incidence data seemed to indicate a treatment dose relationship regarding pulmonary adenomas and adenocarcinomas. Statistical analyses of these data for significance of pulmonary tumor incidence in control and treated male and female mice is presented in Table 1.

Statistical analyses (attached) of combined pulmonary adenomas and adenocarcinomas indicate that male lung tumors were significant for the middle and high treatment dose groups, and high dose females also showed significant lung tumor incidence. A linear, dose related trend for these types of tumors was not evident.

TABLE 1

Total neoplastic and non-neoplastic respiratory system lesions in mice fed Dowco 233 in diets

No. Tumors/No. Examined

Type of Tumor	Study Control & Historical Control	Historical Control	Study Control	24 ppm	80 ppm	240 ppm
MALES	%	%	%	%	%	%
Adenomas	14/150 (9.3)	8/100 (8)	6/50 (12)	14/48 (29)	11/49 (22.4)	15/49 (30.6
Adenocarcinomas	28/150 (18.7)	25/100 (25)	3/50 (6)	2/48 (4.1)	6/49 (12.2)	4/49 (3.[illegible]
Total Tumors/ No. Examined	42/150 (28)	33/100 (33)	9/50 (18)	16/48 (33.3)	17/49 (34.6)	19/49 (38.7
FEMALES	%	%	%	%	%	%
Adenomas	13/150 (8.6)	8/100 (8.6)	5/50 (10)	9/50 (18)	6/48 (12.5)	14/50 (28)
Adenocarcinomas	19/150 (12.6)	17/100 (17)	2/50 (4)	3/50 (6)	4/48 (8.3)	1/50 (2)
Total Tumors/ No. Examined	32/150 (21.3)	25/100 (25)	7/50 (14)	12/50 (24)	10/48 (20.8)	15/50 (30)

Test for Significance of Differences Between Proportions 8/2

Lung Tumors, Females

ppm	# RESP	Total	% +/-2(S.D.)	One Tail P Statistic. Fisher's
0.000	7	50	14.00+/-(10.62)	
24.000	12	50	24.00+/-(12.84)	0.154
80.000	10	48	20.83+/-(12.53)	0.266
240.000	15	50	30.00+/-(13.70)	0.045

Test for a linear trend is not significant

It was noted that the petitioners presented lung tumor incidence (adenomas and adenocarcinomas) for 100 control mice of the same strain from a different chemical toxicity evaluation which ran concurrently with the present test; which they point out have a higher incidence of combined pulmonary tumors, compared with the present study control mice. When these 100 "control" mice are compared with the Dowco 233 mice (below), Dowco 233 treated mice incidence of pulmonary tumors is not significant.

TABLE 2

INCIDENCE OF PULMONARY TUMORS[a] IN CDF_1/COX MICE DURING TWO YEAR STUDIES

Total Pulmonary Tumors	Dowco 233 Control	Concurrent Control	PPM Dowco 233		
			24	30	240
Male	9/50	13/100	16/48	17/49[b]	19/49[b]
Female	7/50	25/98	12/50	10/50	15/50[b]

[a] = Number affected/number examined

[b] = Statistically increased from Dowco 233 control (but not concurrent control) value when analyzed using Fisher's Exact Probability Test. $p < 0.05$.

It is interesting to note that such a wide difference between two groups of the same mouse strain occurred regarding pulmonary tumor incidence; however, this phenomenon suggests that the experiment should be repeated, using larger numbers of control and test mice. Due to the wide variability of spontaneous pulmonary tumor incidence in CDF_1/COX mice, it is unacceptable to include controls from another experiment in the present study.

The results of the present study suggest that a carcinogenic effect was produced in CDF_1/COX mice regarding pulmonary tumors when male and female mice were fed Dowco 233 for 2 years at dose levels of 0, 24, 80, or 240 ppm in the diet. A NOEL was not established for incidence of pulmonary tumors.

Classification - Supplementary Data

(The study should be repeated to clarify the incidence of spontaneous tumors in CDF_1/COX mice).

E. Evaluation of Requested Tolerances:

The petitioner requested tolerances of 1000 ppm triclopyr and its metabolite in or on grasses, forage, and 300 ppm in or on grasses, hay; these items would not contribute directly to the human diet.

RCB recommended that the Petition Section F be changed to show the following requests for human dietary items (these suggested tolerances are tentative, pending results of a lactating goat metabolism study):

0.4 ppm, milk;
1.0 ppm in meat, fat and meat by-products (except kidney and liver);
5.0 ppm in liver and kidney

A provisional ADI based on the 38 day supplemental dog study phenolsulfonthalein (PSP) excretion NOEL of 0.5 mg/kg/day, a PADI may be calculated.

(A PADI based on the dog PSP effects is much lower than a PADI based on decreased body weights found in a 90 day rat study).

A PSP excretion NOEL was not determined for the 6 month dog study; it was shown to be less than 5 mg/kg/day.

PADI based on a NOEL for PSP excretion, 38 day supplemental dog study:

$$\frac{0.5 \text{ mg/kg/day}}{1000 \text{ S.F.}} = 0.0005 \text{ mg/kg/day}$$

A tentative MPI (60 kg person) = 60 x 0.0005 = 0.03 mg/day.

When the tentative tolerances for the use of Garlon recommended by RCB are used (shown above), a total of 957.74% of the PADI would be utilized (printout is attached).

If the Dow Chemical Co. requested tolerances of:

0.1 ppm, milk;

0.1 ppm, in or on meat, fat, and meat by-products (except kidney and liver of cattle, sheep, and goats);

1.0 ppm in or on kidney and liver of liver cattle, sheep, and goats.

are used to calculate the PADI, 183.12% of the PADI would be utilized (printout is attached).

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ADI not recorded per

ACCEPTABLE DAILY INTAKE DATA

Dog	NOEL	S.F.	ADI	DPI
mg/kg	ppm		mg/kg/day	mg/day(60kg)
0.500	20.00	1000	0.0005	0.0300

Current Action PP# 1F2508

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Goats(62)	1.000	0.03	0.00045
Sheep(145)	1.000	0.19	0.00291
Kidney(203)	5.000	0.03	0.00225
Liver(211)	5.000	0.03	0.00225
Milk&Daily Products(93)	0.100	28.62	0.17169
Cattle(26)	1.000	7.18	0.10777

DPI	THRC	% ADI
0.0300 mg/day(60kg)	0.2873 mg/day(1.5kg)	957.74

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RCB-recomendations

ADI recorded per

ACCEPTABLE DAILY INTAKE DATA

Log mg/kg	NOEL ppm	S.F.	ADI mg/kg/day	TPI mg/day(60kg)
0.500	20.00	1000	0.0005	0.0300

Current Action PP# 1F2508

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Goats(82)	0.100	0.03	0.00005
Sheep(145)	0.100	0.19	0.00029
Kidney(203)	1.000	0.03	0.00045
Liver(211)	1.000	0.03	0.00045
Milk&Dairy Products(93)	0.100	28.62	0.04292
Cattle(26)	0.100	7.18	0.01078

TPI	THRC	% ADI
0.0300 mg/day(60kg)	0.0549 mg/day(1.5kg)	183.12

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Co. request